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MALAYAN TROMBIDIID LARVAE, PART I.

(ACARINA: TROMBIDIIDAE)

WITH DESCRIPTIONS OF SEVENTEEN NEW SPECIES.

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(With 16 Figures in the Text.)

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INTRODUCTION.

IN 1915 Dowden reported a suspected case of tsutsugamushi disease, or Japanese river fever, from the Federated Malay States. Twelve years later Fletcher and Field (1927) were able to examine a series of cases and established

the occurrence of this disease in the Malay Peninsula. Meanwhile, Fletcher and Lesslar (1925) had investigated an atypical typhus fever, which they named "tropical typhus," and as a result of further investigation, Fletcher, Lesslar and Lewthwaite (1929) suggested that tropical typhus might be transmitted by a trombidiid larva, in a similar manner to tsutsugamushi disease.

Walch and Keukenschrijver (1924) obtained strong evidence that in Sumatra, tsutsugamushi disease was transmitted by *Trombicula deliensis*, a mite very similar to *T. akamushi*, the proved vector in Japan. In Japan, the reservoir of infection had been found in several rodents, particularly in *Microtus montebelloï*, which, however, does not occur in Malaya. In Formosa, Hatori (1919) suspected that several rodents acted as reservoirs, and in Sumatra, Walch and Keukenschrijver (1924) had incriminated *Rattus rattus diardi* and *Rattus concolor*. Both the latter occur in Malaya, and Fletcher and his co-workers concluded that the common rats acted as reservoirs of tsutsugamushi disease, and probably of tropical typhus as well, in the Malay Peninsula. Confirmation of this view, in relation to tropical typhus, resulted from the researches of Anigstein (1930), and a study of trombidiid larvae parasitic on rats therefore became of considerable importance.

Malayan trombidiid larvae were first studied by Fletcher, Lesslar and Lewthwaite (1928) who examined 130 rats and found four species, including *Trombicula deliensis*, which had previously been recorded by Walch (1923) from Sumatra. On my return from leave in 1928, Dr R. Lewthwaite kindly gave me his collection and a preliminary investigation was begun. This led to a systematic survey of the ecto-parasites of rats, extending over a period of seventeen months in 1929 and 1930, during which over 1200 rats and other small vertebrates were examined. About half the rats came from the town of Kuala Lumpur, and half from an estate and jungle area in the vicinity of Sungei Buloh, including the estate, some twenty miles from Kuala Lumpur, referred to as "Oil-palm Estate" in the writings of Fletcher, Lesslar and Lewthwaite. At first, the examination of rats was limited to the common urban rat (*Rattus rattus diardi*), the rural rat (*Rattus rattus jalorensis*) and the house rat (*Rattus concolor concolor*), but in 1930 other species of *Rattus*, and small animals inhabiting estates, secondary growth and jungle, were included. In addition, several rats were obtained from other districts in the Peninsula.

Over a period of nearly four months, 538 examinations were made of labourers on "Oil-palm Estate," where tsutsugamushi disease had occurred and where tropical typhus was endemic. Eighty-seven trombidiid larvae, belonging to three species, *Trombicula akamushi*, *T. deliensis* and *T. hirsti*, were removed from these labourers. In another district, *T. acuscutellaris* was found attacking man. These species are also parasitic on rats.

Although it is recognised that the larval genera defined by Oudemans (1912 *b*) can only be regarded as temporary, his classification has been followed. As Warburton (1928) points out, *Trombicula*, of all the larval genera, alone possesses any validity, but it is considered better at present to follow

Oudemans's classification than to adopt Warburton's suggestion and include all larval trombidiids in the genus *Leptus*. In following the classification of Oudemans, Ewing (1929) and Sambon (1928) appear to adopt the same view. Berlese and Vitzthum have studied adult trombidiids from the Malaysian region, and some or all of the larval forms here dealt with may prove to have already been described as adults. It will be many years, however, before Malayan trombidiid larvae are correlated with their adults, and meanwhile, owing to their importance as parasites and vectors of disease, easy recognition is desirable.

The present paper is confined to those species which have been found on members of the genus *Rattus* Fischer. Twenty-four species are recorded, of which seventeen are new. Most of the specimens were collected while I was Entomologist to the Institute for Medical Research, Federated Malay States, but I am indebted to Prof. Dr L. Anigstein and Dr A. N. Kingsbury, for arranging to continue collections for me for some time after I had been appointed to Singapore. For facilities on "Oil-palm Estate," I am grateful to Dr G. C. McGregor and to Mr D. W. McCrow and his European Assistants. Dr W. J. Vickers and Dr E. P. G. Ritchie, Health Officers, Kuala Lumpur, kindly provided rats from the town, while Mr M. L. Webber, of the Forest Department, assisted in obtaining Sakai trappers to catch small jungle animals alive. Mr C. Boden Kloss, Director of Museums, generously undertook the determination of the vertebrate hosts, and Mr N. C. E. Miller took infinite trouble over the drawings which, apart from minor alterations, are entirely the product of his pen. Lastly, I wish to express my gratitude to my assistant, A. Ganapathypillai, who mounted all the mites examined and did the preliminary sorting.

Genus **Trombicula** Berlese 1905.

Trombicula Berlese 1905.
Microthrombidium Haller 1882, Oudemans 1912 *b*.
Microthrombidium Oudemans 1909.
Leptotrombidium Nagayo *et al.* 1917 (*Syn.* Nagayo *et al.* 1921).

The only trombidiid larvae which have been reared to the adult stage prove to belong to the genus *Trombicula*, and since they all came within the larval genus *Microthrombidium* as defined by Oudemans (1912 *b*) it is reasonable to assume, as Andrée (1928) suggests, that others belonging to this genus as larvae will prove to be species of *Trombicula* when reared. Hirst (1926 *a*) proposed *Neotrombicula* as a sub-genus for the nymph of "*Leptus autumnalis*."

Some authors write *Thrombicula*, others *Trombicula*. Sambon (1928) states that Fabricius wrote *Trombidium*, rendering it badly from *θρόμβος*, or *θρόμβεῖον*. Andrée (1926) agrees with this derivation, which, he states, is supported by Berlese (1891), and considers that Agassiz (1842) was wrong in giving the derivation as *τρομβώδης*. He therefore considers that the correct spelling should be *Thrombidium*, hence *Thrombicula* and compound names such as

Allothrombium. This spelling is adopted by Oudemans. In the absence of definite evidence of the original derivation, it would appear, in the light of Opinion 34 of the Rules, that the original spelling should be preserved, and *Trombicula* is therefore used in this paper.

Trombicula akamushi (Brumpt).

Trombidium akamushi Brumpt 1910.
Microtrombidium akamushi (Brumpt) Hirst 1915 *a*.
Microtrombidium brumpti Hirst 1915 *a* (*Syn*. Ewing 1920).
Leptotrombidium akamushi (Brumpt) Nagayo *et al*. 1917.
Leptus akamushi (Brumpt) Miyajima and Okumura 1917.
Trombicula coarctata (Berlese) Kitashima and Miyajima 1918.

Originally described as a larva by Brumpt, this species was held by Kitashima and Miyajima to be the larva of *Trombidium coarctatum* Berlese 1888, later amended to *Trombicula coarctata* by Berlese (1912), but the evidence is inconclusive. Coming within the larval genus *Microthrombidium* Haller, according to Oudemans's (1912 *b*) definition, it has been reared to the adult stage by the Japanese workers. Nagayo and his co-workers (1921) apparently make *pseudoakamushi* Tanaka 1916 a synonym of *pallida* Nagayo 1919. Ewing (1925) believes that the four species, *T. pallida*, *T. palpalis*, *T. intermedia* and *T. scutellaris*, described by Nagayo in 1919 and 1920, are merely seasonal variations of *T. akamushi*. Tanaka, Kaiwa, Teramura and Kagaya (1930) recognise *T. pallida* Nagayo and *T. palparis* (? *palpalis*) Nagayo as valid species, however, but name them *T. pseudoakamushi* A Tanaka and *T. pseudoakamushi* B Tanaka respectively. According to these authors, *T. akamushi* (Brumpt) was apparently described by K. Kishida, in 1909, as *Kedania tanakai*, in which case *tanakai* should take precedence as a specific name. Since I have been unable to consult the original paper by Kishida, however, I do not at present propose any change.

During 1929, *T. akamushi* was taken at Sungei Buloh, Selangor, on *Rattus rattus diardi* (Jentink) and *R. rattus jalorensis* (Bonhote) only in March, October and November. The only other animal host was *Trichys fasciculata fasciculata* (Shaw) of Lyon 1907, at Sungei Buloh, Selangor, 1. vi. 30, but it was common on man in the same area, being found continuously on the scrotum of estate labourers over a period of nearly four months from October, 1929 to January, 1930. Fletcher, Lesslar and Lewthwaite (1928) mention having obtained specimens from the same area which "resemble *T. akamushi* even more closely than *T. deliensis*." These specimens were not available to me, but I have no doubt they were *T. akamushi*. Dr H. E. Ewing very kindly examined some of my specimens and confirmed the determination. In view of its prevalence on the human host, it would appear highly probable that this species is the principal vector of tsutsugamushi disease in the Federated Malay States.

Trombicula deliensis Walch.

Trombicula deliensis Walch 1923.
Trombicula deliensis Walch in Fletcher, Lesslar and Lewthwaite 1928.

Dr E. W. Walch kindly furnished me with specimens from Sumatra, with which the Malayan form proves to be identical. For reasons already outlined (Gater, 1930) it is believed that this is a variant of *Trombicula akamushi* (Brumpt), but it is retained as a separate species until more complete evidence has been obtained.

It was very common on *Rattus rattus diardi* (Jentink) and on *R. rattus jalorensis* (Bonhote) in the Sungei Buloh area, Selangor, but was only once taken on a rat from the town of Kuala Lumpur, 20. iv. 30. From Sungei Buloh it was taken in every month during 1929 except June, and it is apparently widely distributed, specimens having been received from Fraser's Hill (4500 ft.), Pahang (Dr A. K. Cosgrave coll.); Raub, Pahang (Mr G. B. Purvis coll.) and Port Dickson, Negri Sembilan (Dr R. M. Forsayeth coll.). It was also found on many other animals, viz.: *Rattus concolor concolor* (Blyth) 1. vii. 29, *R. mülleri validus* (Miller) 24. iv. 30, *R. edwardsi ciliatus* (Bonhote) 25. iv. 30, *R. sabanus vociferans* (Miller) 28. iv. 30, *Trichys fasciculata fasciculata* (Shaw) of Lyon 1907, 12. vi. 30, *Sciurus prevosti humei* Bonhote 1. v. 30, *S. notatus miniatus* Miller 20. iv. 30, *S. nigrovittatus bilimitatus* Miller 16. v. 30, *Rhinosciurus laticaudatus tupaiodes* (Blyth) 10. iv. 30, *Tupaia glis ferruginea* Raffles 27. iii. 30, *Paradoxurus hermaphroditus* (Pallas) sub. sp. 1. v. 30 and *Tragulus kanchil fulviventer* Gray 28. iii. 30. On man it was not nearly as common as *T. akamushi*.

In the original description the number of palpal claws is given as "2 (3)." In Malayan specimens there are three, and Dr Walch (personal communication) informs me that this is the correct number for the Sumatran form.

Trombicula keukenschrijveri Walch.

Trombicula keukenschrijveri Walch 1925.

This species was described from specimens without the pseudostigmatic organs, which are missing from my specimens. Through the kindness of Dr E. W. Walch I have been able to examine his slides and in the absence of pseudostigmatic organs the Malayan and Sumatran forms appear to be identical. Three specimens from *Rattus edwardsi ciliatus* (Bonhote), Fraser's Hill (4500 ft.), Pahang (Dr A. K. Cosgrave coll. 25. iv. 30). Walch's specimens were found on man.

Trombicula hirsti Sambon.

Trombicula hirsti Sambon 1927.
Trombicula pseudoakamushi Hatori in Walch 1923.
Trombicula pseudoakamushi Hatori in Fletcher, Lesslar and Lewthwaite 1928.

Walch (1923) considers that a species occurring in Sumatra is identical with *T. pseudoakamushi* Hatori 1919, but Hatori neither describes nor figures

the species which he names, and *pseudoakamushi* is in any case preoccupied by *T. pseudoakamushi* Tanaka 1916, a form closely allied to, if not identical with *T. akamushi* (Brumpt). Kitashima and Miyajima (1918) refer to a species, sent to them by Hatori from Formosa, which they name *Leptus autumnalis* Shaw, and their figure closely resembles the form recorded by Walch from Sumatra. It is distinct from the *T. autumnalis japonica* described by Tanaka, Kaiwa, Teramura and Kagaya (1930). Kawamura and Yamaguchi (1921) figure and incompletely describe a form which they refer to as *T. pseudoakamushi* Hatori, and which they consider to be the larva of *T. mediocris* Berlese 1912 (which Miyajima, according to Ewing (1920) holds to be the nymph of *T. minor* Berlese 1912). The larva which they figure apparently has the palpal claw trifurcate and is therefore different from the forms dealt with by Walch or Kitashima and Miyajima.

Sambon (1927) described *T. hirsti* on specimens from Australia, and Hirst (1929) suggests that this is identical with the *T. pseudoakamushi* Hatori of Walch. The Sumatran and Malayan forms are the same and correspond with Sambon's description and figures. The species is therefore named *T. hirsti* Sambon.

The confusion in nomenclature possibly accounts for the difference in host relationships noted by Walch (1923). In Formosa, the form designated *T. pseudoakamushi* Hatori by Kawamura and Yamaguchi (1921) is stated never to attack man, but the form described by Walch under this name is found on man in Sumatra as well as in Malaya. *T. hirsti* occurs on man in Australia.

This species was taken on *Rattus rattus diardi* (Jentink) and *R. rattus jalorensis* (Bonhote) in every month during 1929. It was found only once, 27. iv. 29, on a rat from the town of Kuala Lumpur, the other specimens coming from Sungei Buloh, and it was not at any time plentiful. On man it was comparatively rare, being taken in October and December, 1929, on the scrotum of estate labourers in the Sungei Buloh area. It was also found on *Tupaia glis ferruginea* Raffles at Sungei Buloh, 22. vi. 30.

Trombicula acuscutellaris Walch.

Trombicula acuscutellaris Walch 1923.

Dr E. W. Walch kindly forwarded specimens from Sumatra, with which the Malayan form is identical. It was taken on *Rattus rattus diardi* (Jentink) at Sungei Buloh in August, October, November and December, 1929, and in January, 1930, but was only once found on the same sub-species of rat from the town of Kuala Lumpur, 12. viii. 29. Two specimens were taken in Raub, Pahang (Mr G. B. Purvis coll., March, 1930). It was not found on man at Sungei Buloh, nor was it taken on any other animal host, but I have specimens from Setapak, Selangor, taken from a woman school-teacher, who said they attacked her in a rice field (Dr Enid A. Robertson coll., May, 1929).

Trombicula munda n.sp. (Fig. 1).

Body oval, sometimes with a constriction posterior to legs and a fold across body on the posterior portion, in addition to one behind scutum; striations rather weak, slightly crenulated; inconspicuous pitting on scutum and coxae; colour, pale translucent yellow to shrimp pink. *Fangs of chelicerae* short, stout, the tip moderately sharp; dorso-apical tooth minute, ventral tooth apparently absent; maxillary setae[1] plain. *Palpi* rounded; first and second palpal setae plain or with a few short barbs; on fourth segment three setae, plain or with a few barbs of varying length; appendiculum with a prominent pectinate seta; palpal claw bifurcate, sharply pointed. *Scutum* weak, broader than long (mean L^2 35 μ; W 49 μ; pseudostigmatic organs 13 μ apart), trapezoidal, anterior margin practically straight, posterior margin rounded, corners somewhat angular; scutal setae five in number, fine, pilose, the posterior pair the longest, the anterior laterals shortest and placed well back from corners (pseudostigmata and anterior laterals are shown too far forward in the figure). Pseudostigmata slightly in advance of mid-line between setae[3], small; *pseudostigmatic organs* racemiform, slender, with five or six branches. *Ocular shield* apparently absent; a single eye, situated at some distance from the scutum on the mid-line, is visible in some mounts. *Body setae* fifty-four to fifty-six in number, fine, pilose; on dorsum twenty-six, longer anteriorly than posteriorly, arranged in rows approximately as follows: 8 : 6 : 2 : 4 : 4 : 2; on venter variable, longer posteriorly than anteriorly, a group sometimes apparently arranged in parallel rows at sides of a longitudinal fold around anus. *Legs* with terminal segments of I and II abruptly narrowed and a marked constriction between articles 5 and 6 of legs I and II; mean L of fore-leg[4] (ten specimens) 124 μ; leg setae mostly plain or nearly so; coxal setae single, plain, long, fine. *Length*[5] (partially engorged) 0·30–0·41 mm.; *width* 0·21–0·29 mm.

Type host: *Rattus rattus diardi* (Jentink). Locality: Kuala Lumpur, Selangor, Federated Malay States. *Type* and two paratypes in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Numerous specimens from the ears of the type host. Also found on *Rattus mülleri validus* (Miller), Sungei Buloh, Selangor, 8. viii. 30 and on *R. malaisia* Kloss, Sungei Buloh, 6. v. 30. Occurs in small numbers in association with *Neoschöngastia indica* (Hirst), but was taken on *R. rattus diardi* in every month during 1929. It is more common on rats from the town than on those from the country. In one specimen the median anterior scutal seta is duplicated.

[1] The "Galea hairs."

[2] Greatest L (length) and W (width) of scutum.

[3] Between anterior and posterior lateral setae.

[4] Excluding coxa and tarsal claws.

[5] Length from tips of fangs of chelicerae to posterior margin of body: width at broadest portion of body.

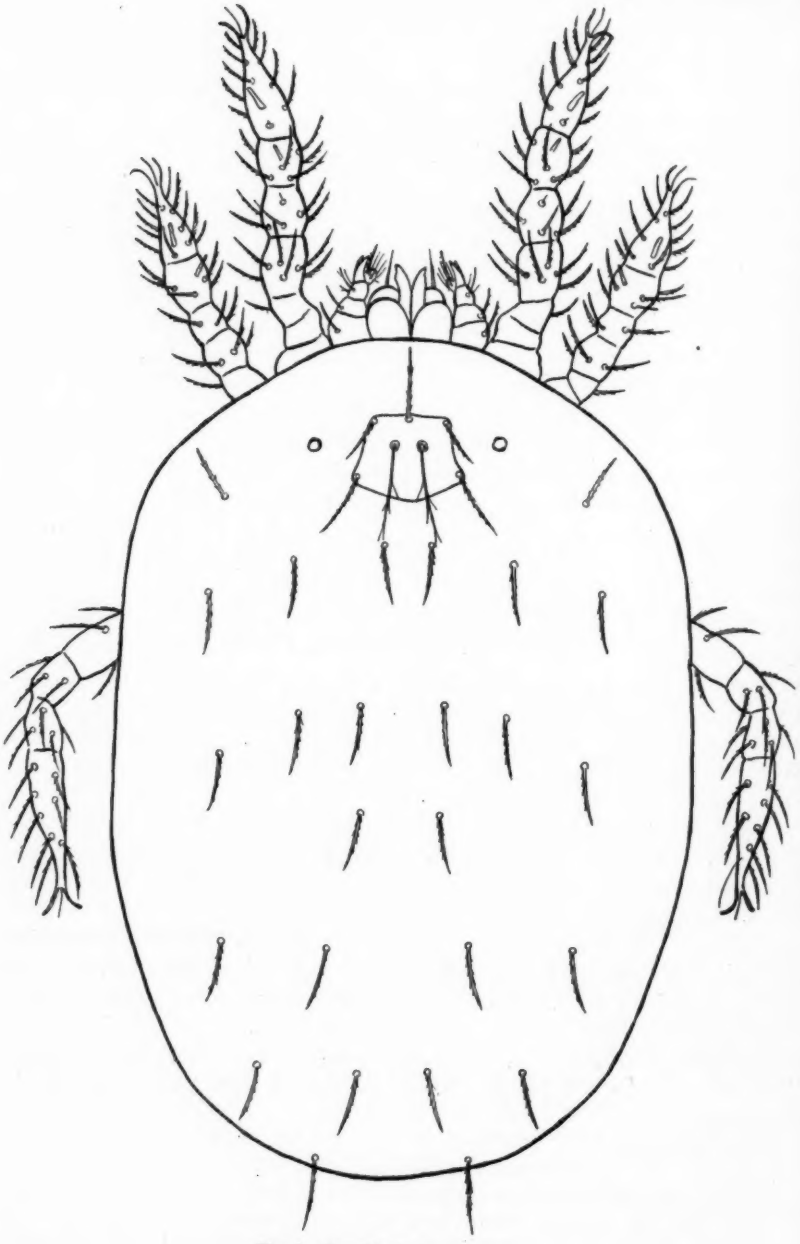

Fig. 1. *Trombicula munda* n.sp

Trombicula spicea n.sp. (Fig. 2).

Body ovoid, a fold across body posterior to hind-legs, sometimes anteriorly as well; striations weak, slightly crenulated; slight pitting on scutum, epistome and coxae. *Fangs of chelicerae* short, rather strongly curved, stout, with a prominent dorsal ridge, tapering abruptly to a fairly sharp point; dorso-apical tooth apparently absent, ventral tooth minute; maxillary setae plain. *Palpi* rounded; first palpal seta with a few minute barbs; second seta plain; on fourth segment three plain setae, the dorsal seta sometimes with a barb; appendiculum with one pectinate seta; palpal claw bifurcate, sharply pointed. *Scutum* broader than long (mean *L* 41 μ; *W* 55 μ; pseudostigmatic organs 14 μ apart), trapezoidal, anterior margin slightly recurved around median seta, corners rounded, posterior margin slightly produced, rounded; scutal setae five in number, pilose, the posterior pair the longest, median and anterior laterals nearly equal, the latter set well back from the corners. Pseudostigmata midway between setae; *pseudostigmatic organs* long, with short, thin stem which thickens distally and carries prominent setules, giving it the appearance of a brush or ear of wheat. *Ocular shield* absent; a single eye, level with pseudostigmata and at some distance from the scutum. *Body setae* fifty or fifty-six in number, pilose; on dorsum twenty-six, arranged in rows approximately as follows: 8 : 4 : 4 : 4 : 4 : 2; on venter variable, longer posteriorly than anteriorly. *Legs* with terminal segments tapering somewhat abruptly; mean *L*, fore-leg, 137 μ; leg setae mostly plain or only slightly barbed; coxal setae single, fine, plain or with one or two fine barbs. *Length* (partially engorged) 0·29–0·33 mm.; *width* 0·20–0·23 mm.

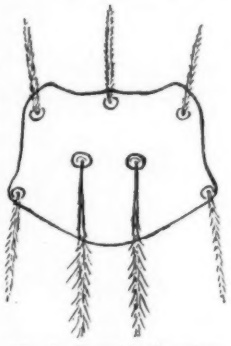

Fig. 2. *Trombicula spicea* n.sp. Scutum.

Type host: *Rattus malaisia* Kloss. Locality: Sungei Buloh, Selangor, Federated Malay States. *Type* in British Museum; paratypes in United States National Museum and King Edward VII College of Medicine, Singapore.

Five specimens from the ear of the type host, 8. viii. 30. Also found on *Rattus mülleri validus* (Miller), Sungei Buloh, Selangor, 8. viii. 30. This species is apparently related to *T. munda*, but differs from it in the form of the scutum and pseudostigmatic organs.

Trombicula hastata n.sp. (Figs. 3 and 4).

Body ovoid, rounded; striations moderate, slightly crenulated; pitting on scutum, epistome and legs. *Fangs of chelicerae* moderately stout, slightly curved, abruptly tapering to a sharp point which appears to be set at an angle; dorso-apical and ventral teeth apparently absent; maxillary setae strongly pectinate. *Palpi* rounded; first and second palpal setae short, plain; on fourth segment the dorsal seta with a few short barbs, the other two

plain; appendiculum with six pectinate setae; palpal claw long, trifurcate, sharply pointed, the upper accessory prong longer than the lower. *Scutum* broader than long (mean *L* 44 μ; *W* 71 μ; pseudostigmatic organs 37 μ apart), trapezoidal, anterior margin concave, posterior margin slightly concave, corners rounded; scutal setae five in number, median anterior small, lanceolate and placed well back from margin; anterior laterals short, stout, strongly pectinate, situated on corners; posterior laterals large, lanceolate and situated under two-thirds the length of scutum from anterior pair. Pseudostigmata behind posterior lateral setae, as far in front of the posterior margin of the

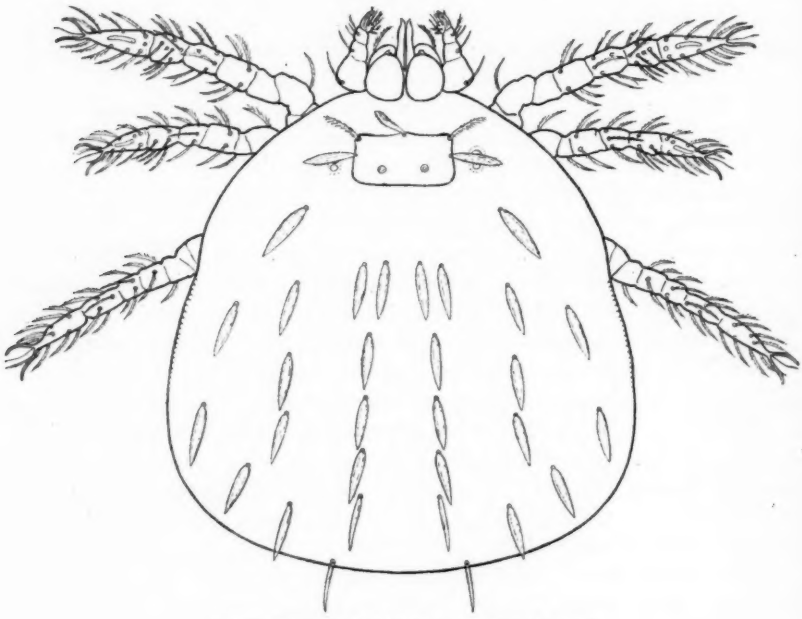

Fig. 3. *Trombicula hastata* n.sp. Dorsal aspect.

scutum as the distance between the anterior and posterior lateral setae; *pseudostigmatic organs* missing in these specimens. *Body setae* large, forty-eight in number; on dorsum thirty, large, translucent, lanceolate, covered with minute hairs, becoming smaller posteriorly, but two pairs of the usual type of body seta, stout, minutely pilose, on posterior edge of body; on venter of the usual type, strongly serrate to pectinate, much longer posteriorly than anteriorly. *Fore-leg*, mean *L* 157 μ; leg setae mostly serrate or pectinate with a few plain; coxal setae single, serrate or strongly pectinate, the one on the mid-coxa the shortest. *Length* (partially engorged) 0·42–0·46 mm.; *width* 0·26–0·35 mm.

Type host: *Rattus surifer surifer* (Miller). Locality: Sungei Buloh, Selangor,

Federated Malay States. *Type* and paratype in British Museum; paratype in King Edward VII College of Medicine, Singapore.

Described from three specimens from the ear of the type host, 19. v. 30. As far as I am aware this species, owing to the peculiar form of the dorsal setae, which slightly resemble those found in *Achorolophus* (Erythraeidae), is

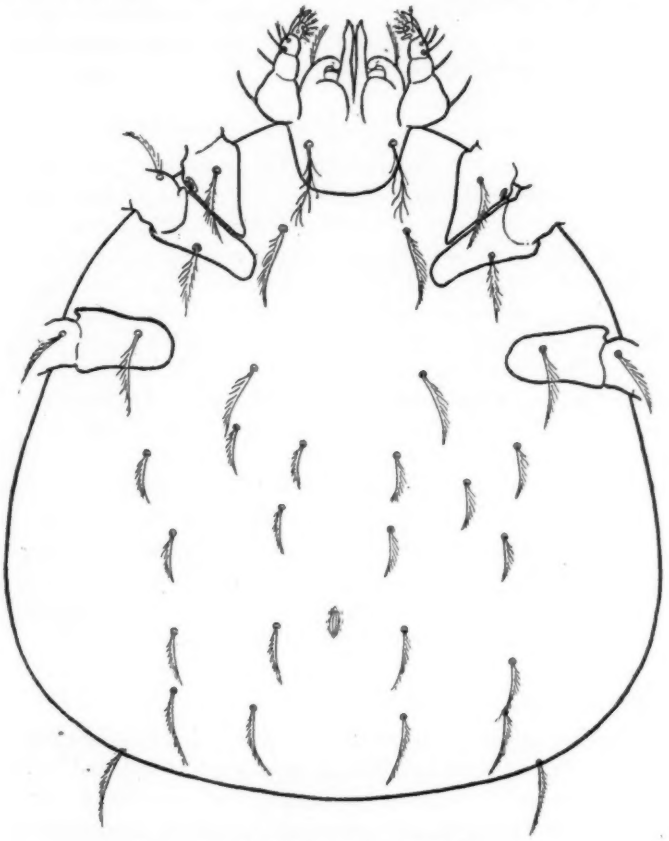

Fig. 4. *Trombicula hastata* n.sp. Ventral aspect.

distinct from any other described. In the absence of pseudostigmatic organs I place it provisionally in the genus *Trombicula*.

Genus **Schöngastia** Oudemans 1910.

Schöngastia Oudemans 1910 *a*.

Ewing (1929) has divided this genus into two, forming *Neoschöngastia* to include those species which do not possess a row of teeth dorsally on the fangs of the chelicerae. Walch (1927) wishes to sink *Schöngastia* as a sub-genus

of *Trombicula* and (1924) brings some experimental evidence to support his view. As Sambon (1928) points out, the experiments are open to criticism, and Ewing's amended definition of the genus is here accepted. Since, however, *Neoschöngastia* only differs from *Trombicula* in the form of the pseudostigmatic organs, further investigation may prove Walch's contention to be correct as far as this genus is concerned.

Schöngastia oudemansi (Walch).

Trombicula oudemansi Walch 1923.
Trombicula oudemansi Walch in Fletcher, Lesslar and Lewthwaite 1928.

Dr E. W. Walch kindly provided me with specimens from Sumatra for comparison with the Malayan form. It was taken on *Rattus rattus diardi* (Jentink) and *R. rattus jalorensis* (Bonhote), from Sungei Buloh, throughout the year with the exception of May and August. It was never taken on man, nor on rats from the town of Kuala Lumpur. In 1930 it was found on the following animals: *Rattus mülleri validus* (Miller) 24. iv, *Trichys fasciculata fasciculata* (Shaw) of Lyon 1907, 12. vi, *Sciurus notatus miniatus* Miller 14. iv, *Rhinosciurus laticaudatus tupaiodes* (Blyth) 10. iv, *Tupaia glis ferruginea* Raffles 10. vi and *Tragulus kanchil fulviventer* Gray 28. iii from Sungei Buloh; on *Rattus edwardsi ciliatus* (Bonhote) from Fraser's Hill (4500 ft.), Pahang (Dr A. K. Cosgrave coll. 25. iv).

Although conforming in other respects to Ewing's amended definition of *Schöngastia*, this species is exceptional in having only six articles in the mid- and hind-legs. If other species are found with the same combination of characters, generic separation may be necessary. As a species of *Schöngastia*, *oudemansi* is valid, but as a species of *Trombicula* the name is preoccupied by *Microtrombidium oudemansi* von Goosmann 1917.

Schöngastia vieta n.sp. (Fig. 5).

Body oval; striations prominent, strongly crenulated, appendages highly sclerotised; pitting on scutum, epistome, hypostome, coxae and legs; colour, reddish pink. *Fangs of chelicerae* stout, moderately curved, a row of teeth dorsally from near the tip to about the middle, ventral tooth possibly represented by an exceedingly minute indentation in some specimens; maxillary setae plain, maxillary portion of the hypostome long, often projecting beyond fangs. *Palpi* tending to angulation; first palpal seta with a few barbs or pectinate, variable; second palpal seta long, plain or with a few barbs; on fourth segment a plain seta dorsally and laterally, and a stout, pectinate seta ventrally; appendiculum with five or six pectinate setae, one of which is stout; palpal claw trifurcate, sharply pointed, accessory prongs unequal, the lower accessory as long as or longer than main element. *Scutum* broader than long (mean *L* 56μ; *W* 76μ; pseudostigmatic organs 20μ apart), trapezoidal, anterior margin recurved around median seta, which is set well back, posterior

edge rather deeply convex, tending to form an angle with the appearance of lamellae at its point, corners angular; scutal setae five in number, strongly pectinate or plumose, anterior laterals the longest, median very short. Pseudostigmata posterior to mid-line, slightly in advance of posterior lateral setae; *pseudostigmatic organs* capitate, flask-shaped, the stem moderately long, the enlarged head clothed in minute setules; *ocular shield* placed its own width away from scutum, level with posterior scutal setae, anterior eye the larger. *Body setae* stout, strongly pilose to pectinate, forty-eight to sixty-four in

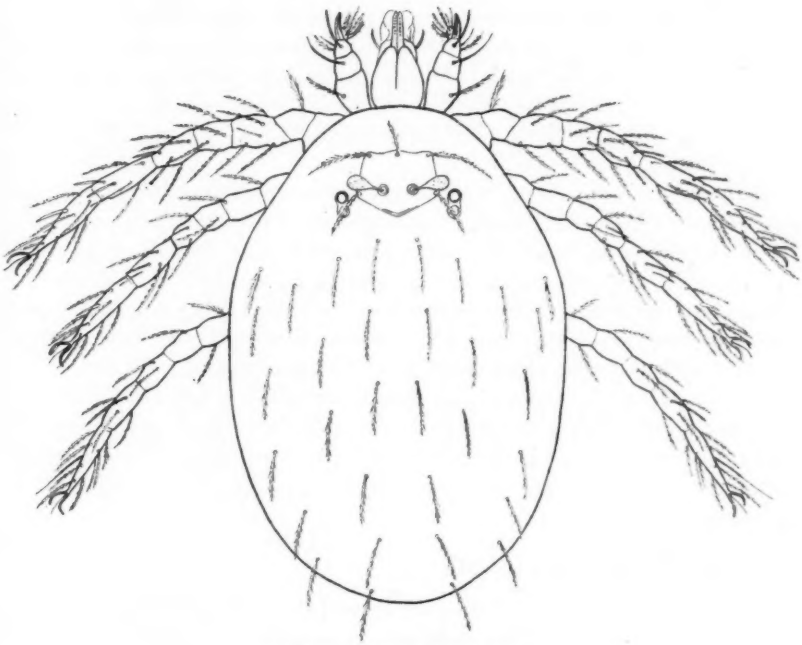

Fig. 5. *Schöngastia vieta* n.sp.

number; on dorsum, thirty-two, arranged approximately in rows as follows: 10 : 6 : 6 : 4 : 4 : 2; on venter the anterior setae shorter and more strongly pectinate than the posterior. *Fore-leg*, mean *L* 243 μ; leg setae mainly pectinate or plumose, but a long, fine, plain seta on article seven of leg III; coxal setae single, strongly pectinate or plumose. *Length* 0·23 (unengorged) to 0·49 mm. (engorged); *width* 0·22 (unengorged) to 0·35 mm. (engorged).

Type host: *Rattus rattus diardi* (Jentink). Locality: Kuala Lumpur, Selangor, Federated Malay States. *Type* and three paratypes in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Ten specimens from the ears of the type host, 17. ii. 30 and *R. rattus*

jalorensis (Bonhote), from Sungei Buloh, Selangor, 22. ii. 30. Also found on *Rattus mülleri validus* (Miller) from Raub, Pahang (Mr G. B. Purvis coll. 5. v. 30).

Genus **Neoschöngastia** Ewing 1929.

Neoschöngastia indica (Hirst).

Schöngastia indica Hirst 1915 *b*.
Trombicula muris Walch 1923 (*Syn.* Walch 1927).
Trombicula muris Walch in Fletcher, Lesslar and Lewthwaite 1928.

This is by far the commonest species on *Rattus rattus*, whether from the town or country, and was taken in large numbers on the two sub-species, *diardi* (Jentink) and *jalorensis* (Bonhote) throughout the year. It was also found on *Rattus concolor concolor* (Blyth) 27. iv. 29 and 4. x. 29 from Kuala Lumpur, *R. sabanus vociferans* Miller, Sungei Buloh 28. iv. 30 and *R. malaisia* Kloss, Sungei Buloh 6. v. 30.

This species is apparently restricted in its host relationships, but is very common and widely distributed. I have specimens from Port Dickson, Negri Sembilan (Dr R. M. Forsayeth coll.); from Seremban, Negri Sembilan (Mr G. B. Purvis coll.) and Raub, Pahang (Mr G. B. Purvis coll.). It remains attached for some time after the death of its host, having frequently been found on rats which had died some twelve hours prior to examination. A specimen from Kuala Lumpur has two setae on one coxa.

Among typical examples of this species, generally in the same cluster, a form having twenty-four to twenty-six dorsal body setae, as opposed to the thirty-two to thirty-four in the typical form, is often found. Since no other differences could be found, however, I regard this merely as a variation of *S. indica*.

Neoschöngastia lacunosa n.sp. (Fig. 6).

Body oval, usually appearing as if stippled; striations prominent, strongly crenulated; pitting on scutum, epistome, hypostome, legs and palpi; appendages strongly sclerotised; colour, greenish white. *Fangs of chelicerae* stout, sharply pointed; dorso-apical tooth prominent, directed backwards, very sharp, with an indentation behind; ventral tooth arising from a ridge, directed backwards; maxillary setae plain. *Palpi* rounded, sometimes tending to angulation; first palpal seta plain or with a few serrations; second palpal seta plain, very long; on fourth segment the dorsal seta minutely barbed or pectinate, lateral and ventral setae plain or with a few barbs; appendiculum with five or six pectinate setae, one of which is stout; palpal claw bifurcate, stout, the accessory prong over three-quarters the length of main element. *Scutum* broader than long (mean *L* 55 μ; *W* 78 μ; pseudostigmata 37 μ apart), trapezoidal, anterior margin strongly recurved around median seta, posterior margin rather sharply concave in centre, corners somewhat angular anteriorly, rounded posteriorly; scutal setae five in number, coarsely pilose or serrate,

posterior pair the shortest, median anterior the longest. Pseudostigmata below mid-line between scutal setae; *pseudostigmatic organs* capitate, spindle-shaped, compressed, the margins sometimes asymmetrical, very transparent, stem short, the head covered with minute setules; *ocular shield* level with posterior lateral setae, slightly over its own width away from scutum, the

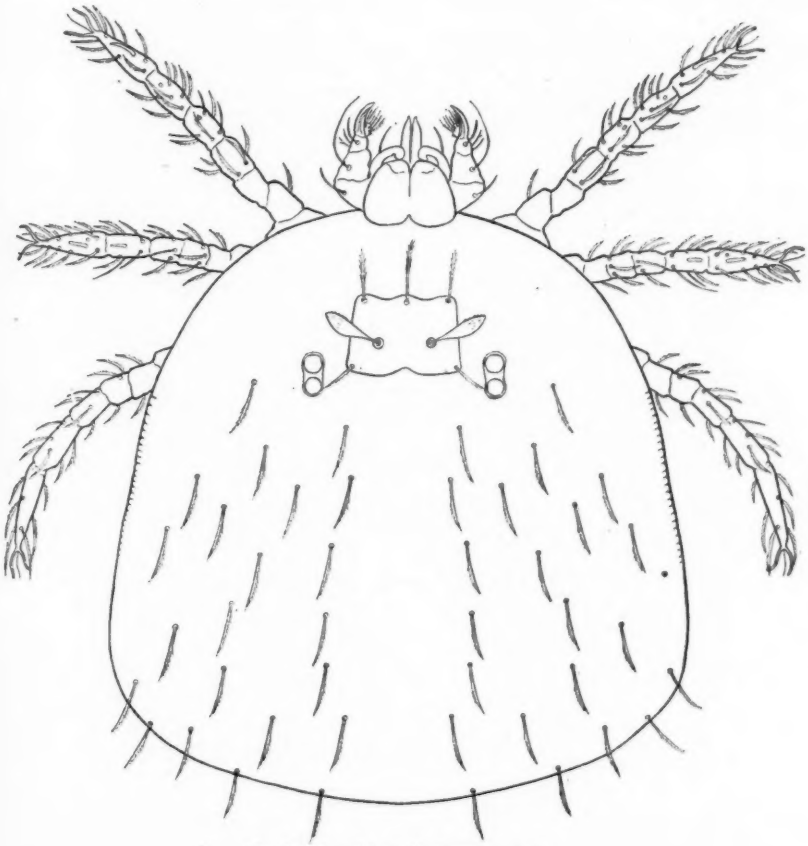

Fig. 6. *Neoschöngastia lacunosa* n.sp.

anterior eye the larger. *Body setae* variable, eighty-six to one hundred and twelve in number, long, stout, coarsely pilose; on dorsum forty-four to fifty-two, showing great variation in arrangement and not to be classified in arbitrary rows; on venter longer posteriorly than anteriorly, pilose, variable in number and position. *Fore leg*, mean *L* (ten specimens) 234 μ; leg setae mostly minutely serrate or slightly pectinate, with some plain setae; coxal setae single, plain to slightly serrate or weakly pectinate. *Length* (partially engorged) 0·49–0·68 mm.; *width* 0·30–0·46 mm.

Type host: *Rattus sabanus vociferans* (Miller). Locality: Sungei Buloh, Selangor, Federated Malay States. *Type* and three paratypes in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Twenty-three specimens from the ears of the type host, 9. vi. 30 and 23. vii. 30.

Neoschöngastia malayensis n.sp. (Fig. 7).

Body round to oval; striations very weak, scarcely crenulated; pitting weak, on scutum, epistome, hypostome and coxae. *Fangs of chelicerae* stout,

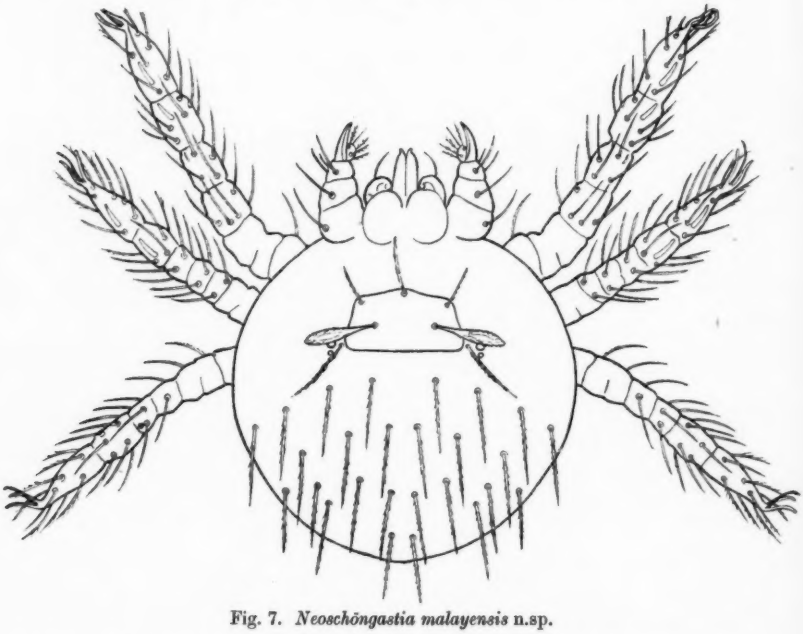

Fig. 7. *Neoschöngastia malayensis* n.sp.

short, slightly curved; dorso-apical tooth minute, directed backwards, very sharp, with a notch behind, ventral tooth arising from a ridge, directed backwards; maxillary setae plain. *Palpi* rounded; first palpal seta plain, second palpal seta plain or with one or two barbs; on fourth segment three setae, plain or with one or two barbs; appendiculum with four pectinate setae, one of which is stout; palpal claw bifurcate, the accessory prong over three-quarters the length of main element. *Scutum* broader than long (mean *L* 34 μ; *W* 50 μ; pseudostigmata 22 μ apart), trapezoidal, anterior margin slightly convex, very slightly recurved around median seta, sides straight until they curve to form broadly rounded posterior corners, posterior margin straight; only the three anterior scutal setae placed on the scutum, anterior laterals

set back from the corners, posterior laterals outside the scutum; scutal setae minutely pilose, posterior pair the longest, anterior laterals the shortest. Pseudostigmata anterior to mid-line between scutal setae; *pseudostigmatic organs* spindle-shaped, the stem long, covered with exceedingly minute setules. *Ocular shield* very weak or obsolete, eyes small, the anterior slightly the larger. *Body setae* stout, long, minutely pilose, shorter posteriorly than anteriorly, forty-eight to fifty in number; on dorsum twenty-six, arranged in rows approximately as follows: 8 : 6 : 6 : 4 : 2; on venter finer than those above, shorter anteriorly than posteriorly. *Legs* with terminal segments tapering abruptly; mean *L*, fore-leg, 135 μ; leg setae mostly plain or slightly serrate except a group on the last article of leg III, which are slightly pectinate; coxal setae single, fine, plain. *Length* 0·18 mm. (unengorged) to 0·39 mm. (partially engorged); *width* 0·13 mm. (unengorged) to 0·25 mm. (partially engorged).

Type host: *Rattus malaisia* Kloss. Locality: Sungei Buloh, Selangor, Federated Malay States. *Type* and paratype in British Museum; paratypes in United States National Museum and King Edward VII College of Medicine, Singapore.

Five specimens from the ears of the type host, 24. vi. 30. This species is at once distinguished by the arrangement of the scutal setae, which is unlike that of any other known Malayan form, but is similar in this respect to *Schöngastia aethiopica* Hirst 1926 *b*, which, since the fangs are not described, may also prove to be a member of the genus *Neoschöngastia*.

Neoschöngastia mutabilis n.sp.

Body oval, striations weak, barely crenulated; pitting on scutum and legs. *Fangs of chelicerae* short, stout, strongly curved, sharply pointed, dorso-apical tooth minute, very sharp, directed backwards, ventral tooth absent; maxillary setae plain. *Palpi* rounded; first and second palpal setae with a few barbs; on fourth segment the dorsal seta long, plain or with a few barbs, lateral seta plain, ventral seta with a few barbs and rather longer and stouter than usual; appendiculum with five pectinate setae; palpal claw trifurcate, the lower accessory prong shorter than the upper. *Scutum* broader than long (mean *L* 45 μ; *W* 72 μ; pseudostigmatic organs 12 μ apart), trapezoidal, anterior and lateral margins concave, posterior margin evenly rounded; scutal setae five in number, long, coarsely pilose to pectinate, anterior laterals usually the longest, median anterior shortest. Pseudostigmata below mid-line between setae, almost level with posterior pair, on either side of a thickened ridge on scutum; *pseudostigmatic organs* capitate, the stem thin and short, the head almost globular, covered with exceedingly minute setules. *Ocular shield* apparently absent, one large eye a little in advance of the level of pseudostigmata. *Body setae* eighty to ninety in number, stout, coarsely pilose to pectinate; on dorsum forty to forty-two, arranged in rows approximately as follows: 10 : 6 : 6 : 8 : 2 : 4 : 2 : 2; on venter much longer posteriorly than anteriorly.

Legs with terminal segments of I and II tapering abruptly; mean *L*, fore-leg, 212 μ; leg setae serrate to pectinate; coxal setae serrate to pectinate, single on I and II, three to five on each of III, the number varying on two coxae of the same specimen. *Length* (partially engorged) 0·39–0·57 mm.; *width* 0·23–0·34 mm.

Type host: *Rattus sabanus vociferans* (Miller). Locality: Sungei Buloh, Selangor, Federated Malay States. *Type* and paratype in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Ten specimens in one cluster in the ear of the type host, 23. vii. 30. This species is very similar to "*Trombidium*" *globulare* Walch 1927, specimens of which I have been privileged to examine through the kindness of Dr E. W. Walch. It differs mainly in the form of the fangs, in the palpal claw, which is trifurcate instead of bifurcate, and in the pseudostigmata being slightly in advance of the posterior lateral scutal setae instead of behind them. Other minor differences occur. The variation in the number of coxal setae appears somewhat remarkable, but since it occurs in the same specimen and all examples were collected from the same cluster, it would appear to be a normal characteristic of this form.

Neoschöngastia debilis n.sp. (Fig. 8).

Body oval; striations faint, weakly crenulated; pitting weak on scutum, epistome and coxae. *Fangs of chelicerae* short, stout, dorso-apical tooth represented only by a swelling, ventral tooth absent; maxillary setae with one or two barbs. *Palpi* slightly angulated; first and second palpal setae plain; on fourth segment the dorsal and lateral setae slightly pectinate, the ventral seta plain; appendiculum with four slightly pectinate setae; palpal claw trifurcate, the accessory prongs equal and two-thirds as long as main element. *Scutum* broader than long (*L* 24 μ; *W* 49 μ; pseudostigmata 17 μ apart), almost rectangular, anterior margin practically straight, lateral margins very slightly concave, posterior margin straight; scutal setae five in number, short, strongly pectinate, posterior pair the longest, anterior laterals the shortest. Pseudostigmata large, on mid-line between scutal setae; *pseudostigmatic organs* damaged in the specimen, one broken stem suggesting a capitate form; *ocular shield* and eyes apparently absent. *Body setae* short, strongly pectinate, ninety in number; on dorsum fifty-four, arranged in rows approximately as follows: 14 : 4 : 8 : 7 : 10 : 2 : 5 : 4; on venter shorter than on dorsum, longer posteriorly than anteriorly. *Legs* with terminal segments tapering abruptly; *L*, fore-leg, 135 μ; leg setae mostly strongly pectinate or barbed; coxal setae single, stout, strongly pectinate, those on coxae II less so than the others. *Length* (partially engorged) 0·34 mm.; *width* 0·26 mm.

Fig. 8. *Neoschöngastia debilis* n.sp. Scutum.

Type host: *Rattus cremoriventer cremoriventer* (Miller). Locality: Sungei Buloh, Selangor, Federated Malay States. *Holotype* in British Museum.

A single specimen from the ear of the type host, 2. viii. 30. The form of the scutum at once distinguishes this species from any other known Malayan form. In the absence of complete pseudostigmatic organs I place it provisionally in the genus *Neoschöngastia*.

Genus **Gahrliepia** Oudemans 1912.

Gahrliepia Oudemans 1912 *a*.
Typhlothrombium Oudemans 1910 *b*.
Schöngastiella Hirst 1915 *b*, n.syn.

Gahrliepia was proposed by Oudemans, since *Typhlothrombium* Oudemans was preoccupied by *Typhlothrombium* Berlese for an adult genus. *Typhlothrombium* Oudemans was founded on a single species, *T. nanus* Oudemans 1910 *b*, and as far as can be ascertained, no other species has since been attributed to this genus. Hirst proposed *Schöngastiella* to include *S. bengalensis* Hirst 1915 *b*, a species very similar to *T. nanus*, except that there are three pairs of setae (in addition to the pseudostigmatic organs) on the scutum, instead of four. Subsequently, Oudemans (1929) described *Schöngastiella disparunguis*.

The Malayan fauna appears to be particularly rich in forms coming within Oudemans's definition of *Gahrliepia* (*Typhlothrombium*) except for the number of scutal setae, and six species, all showing a remarkable agreement in other characters, are here described. The close similarity of these forms, and the fact that the number of scutal setae, posterior to the first two pairs, varies in different specimens of *G. fletcheri* from the same cluster, leads me to believe that the number of scutal setae is not a sound criterion for the separation of genera in this group. I propose, therefore, to include the six species here described, together with those attributed to the genus *Schöngastiella*, in a single genus. All these agree in the form of the scutum and pseudostigmatic organs (in *G. rustica* and *G. turmalis* the posterior portion of the scutum is reduced but still salient), in the general form of the palpi and in having only six articles in the mid- and hind-legs. The Malayan species differ, from those previously described, in having the palpal claw trifurcate instead of bifurcate, but the accessory prongs are usually so fine, and so closely adpressed to the main element, that the separation might easily be missed in the examination of a single specimen. In *G. nanus* eyes are said to be absent, while they were observed in both species of *Schöngastiella*. In the present series eyes were visible in four out of the six species, but in many cases became invisible after the specimens had been mounted for some time.

Gahrliepia fletcheri n.sp. (Fig. 9).

Body oval; striations usually prominent, wavy, following contour of large scutum; moderately crenulated; appendages highly sclerotised; pitting on scutum, epistome, hypostome and legs; colour, greenish yellow. *Fangs of*

chelicerae short, stout, strongly curved ventrally, finely pointed; dorso-apical tooth prominent, blunt, ventral tooth arising from a ridge, minute, backwardly directed; maxillary setae plain. *Palpi* strongly angulated with a small tubercle at point of angulation; first palpal seta with only a few barbs, or pectinate at base, varying even in the same individual; second palpal seta plain, or with one or two barbs; on fourth segment three plain setae; appendiculum with three pectinate setae and a stout, plumose seta; palpal claw

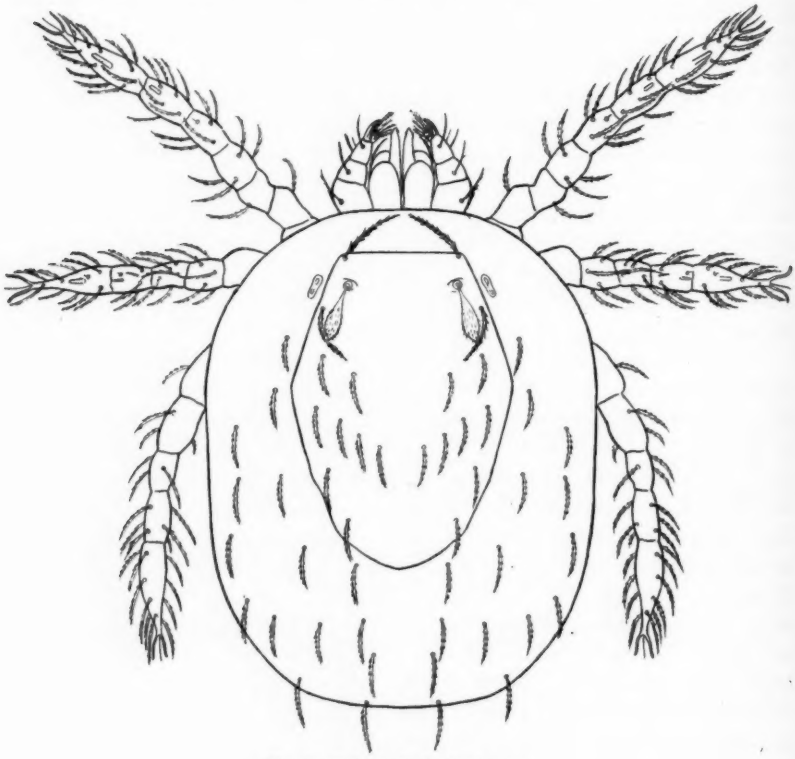

Fig. 9. *Gahrliepia fletcheri* n.sp.

trifurcate, accessory prongs closely adpressed to main element, the lower accessory shorter than the upper. *Scutum* tongue-shaped, longer than broad (mean *L* 189μ; *W* 99μ; pseudostigmatic organs 50μ apart), broadest just before the middle, rather variable in shape (the sides at the greatest breadth are shown to be somewhat too angular in the figure); prominent pits, as well as minute ones, are present. Scutal setae variable in number from fourteen to twenty and of two forms; the two anterior pairs, placed at the anterior lateral angles, and on the sides posterior to the pseudostigmata, respectively, are stout and very coarsely pilose, almost pectinate; the other scutal setae

are more slender and slightly serrate, varying in number, the number on each side of the mid-line of the scutum often differing. Commonly, there are eighteen scutal setae, those situated posteriorly to the first two pairs being arranged in approximate rows, but variations occur. Pseudostigmata on mid-line between first two pairs of scutal setae or just posterior to it; *pseudostigmatic organs* capitate, spindle-shaped, the head pointed distally and tapering gradually into the short stem proximally, covered with fine but prominent setules. *Ocular shield* level with the pseudostigmata, reduced; eyes reduced, irregularly oval in outline, the anterior the larger; both shield and eyes are usually only clearly visible in freshly mounted specimens. *Body setae* above coarsely pilose, thirty to thirty-two in number, arranged approximately as follows: 1 : 2 : 2 on each side of scutum, then 6 : 6 : 4 : 4 : 2 across body; on venter much shorter and finer, fifty to sixty in number, irregularly arranged, serrate to pectinate, much shorter anteriorly than posteriorly. *Legs* with terminal segments tapering abruptly; mean *L*, fore-leg (ten specimens), 172 μ; leg setae mostly serrate or with one or more barbs; coxal setae single, long, serrate or pectinate. *Length* 0·32–0·46 mm.; *width* 0·24–0·33 mm.

Type host: *Rattus rattus diardi* (Jentink). Locality: Kuala Lumpur, Selangor, Federated Malay States. *Type* and two paratypes in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Described from numerous specimens from the ears of the type host. Taken in March, April, May, June, July, August and November, 1929. Also found at Sungei Buloh, Selangor, on *Rattus sabanus vociferans* Miller 9. vi. 30, *Sciurus caniceps concolor* Blyth 19. v. 30, *Tupaia glis ferruginea* Raffles 22. v. 30 and *Rhinosciurus tupaiodes laticaudatus* (Blyth) 29. v. 30.

It is more prevalent on rats from the town than on those from the country and exhibits a reluctance to leave its host, having been the only trombidiid found on some rats which had been dead for twelve hours or more when received. This is a very variable species, but since the extremes of variation can be found among specimens from the same cluster and in some cases in the same individual, it would be unjustifiable to form more than one species. The first examples I saw were taken by Dr R. Lewthwaite while investigating tropical typhus with Dr William Fletcher, after whom the species is named.

Gahrliepia ciliata n.sp. (Fig. 10).

Body ovoid; striations prominent, following contour of scutum dorsally, slightly crenulated; fine pitting on scutum, epistome, hypostome, coxae and legs; appendages strongly sclerotised. *Fangs of chelicerae* short, stout, strongly curved ventrally, dorso-apical tooth blunt, ventral tooth arising from a ridge, prominent, backwardly directed; maxillary setae plain. *Palpi* angulated; first and second palpal setae plain; on fourth segment three plain setae; appendiculum with four pectinate setae and a stout, plumose seta; palpal

claw trifurcate, slender, lower accessory prong shorter than upper. *Scutum* longer than broad (*L* 187 μ; *W* 109 μ; pseudostigmata 42 μ apart), tongue-shaped, anterior margin straight, lateral margins slightly constricted posterior to row of four scutal setae, posterior portion bluntly rounded. Pseudostigmata on mid-line between the two anterior pairs of setae; *pseudostigmatic organs* missing in the specimen, but probably capitate, spindle-shaped; scutal setae long, fourteen in number, anterior two pairs coarsely pilose, posterior pairs more finely pilose or serrate; one pair anterior to pseudostigmata, one pair posterior to them, then in rows across scutum as follows: 4 : 2 : 2 : 2; the last three pairs situated just within the margins of scutum. *Ocular shield* weak; eyes present, irregularly oval, the anterior eye the larger. *Body setae* sixty-

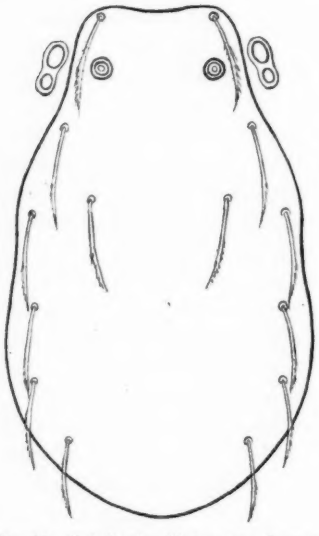

Fig. 10. *Gahrliepia ciliata* n.sp. Scutum.

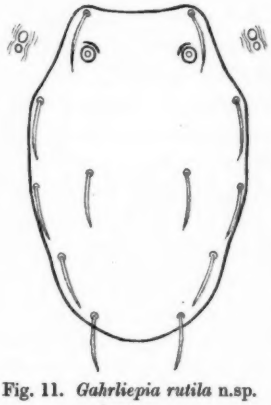

Fig. 11. *Gahrliepia rutila* n.sp. Scutum.

four in number; on dorsum twenty-six, stout, long, coarsely pilose, almost pectinate, longer anteriorly than posteriorly, arranged 1 : 1 : 1 : 1 on each side of scutum and then across body in rows approximately as follows: 6 : 4 : 2 : 4 : 2; on venter small, fine, more finely pilose than on dorsum, slightly longer posteriorly than anteriorly. *Legs* with terminal segments tapering abruptly; *L*, fore-leg, 189 μ; leg setae slightly serrate to pectinate; coxal setae single, serrate to pectinate. *Length* 0·38 mm.; *width* 0·26 mm.

Type host: *Rattus mülleri validus* (Miller). Locality: Sungei Buloh, Selangor, Federated Malay States. *Holotype* in British Museum.

A single specimen from the ear, 8. viii. 30. This species is apparently closely related to *G. fletcheri*, but differs from it in the form of the scutum, the number and arrangement of setae on the dorsum and in the form of the palpal setae.

Gahrliepia rutila n.sp. (Fig. 11).

Body ovoid, extremity pointed, sometimes with folds across centre; striations faint, barely crenulated; pitting weak, on scutum, epistome, hypostome and legs; appendages highly sclerotised. *Fangs of chelicerae* stout, strongly curved below, dorso-apical tooth very prominent, blunt, ventral tooth arising from a ridge, small, directed backwards; maxillary setae plain. *Palpi* moderately angulated; first and second palpal setae short, plain; on fourth segment three plain setae, the dorsal one long; appendiculum with four pectinate setae, one of which is fairly stout; palpal claw trifurcate, fine, the lower accessory prong shorter than the upper. *Scutum* longer than broad (mean *L* 127 μ; *W* 88 μ; pseudostigmata 41 μ apart), tongue-shaped, anterior margin slightly concave; slightly constricted just below the row of four scutal setae, extremity bluntly pointed; scutal setae twelve in number, fine, short, plain or slightly serrate, a pair situated at anterior corners of scutum, a pair posterior to pseudostigmata near lateral margins, a row of four across the scutum and two pairs near margins, the posterior pair near the extremity of scutum. Pseudostigmata slightly below mid-line between first two pairs of setae; *pseudostigmatic organs* missing in all specimens, but probably of the usual form in this genus (spindle-shaped). *Ocular shield* reduced or absent; eyes small, oval, the anterior the larger, situated three times their own width away from scutum, level with pseudostigmata. *Body setae* short, fine, plain or very slightly serrate, fifty-six to sixty-six in number; on dorsum twenty-four to twenty-six, longer anteriorly than posteriorly, arranged 1 : 1 : 1 on each side of scutum, then across body in approximate rows as follows: 6 : 4 : 4 : 4 : 2; on venter longer posteriorly than anteriorly. *Legs* with terminal segments abruptly narrowed; mean *L*, fore-leg, 153 μ; leg setae plain or very slightly serrate, a few pectinate on terminal segments; coxal setae single, moderately long, serrate to pectinate. *Length* (partially engorged) 0·32–0·36 mm.; *width* 0·21–0·26 mm.

Type host: *Rattus mülleri validus* (Miller). Locality: Sungei Buloh, Selangor, Federated Malay States. *Type* and paratype in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Seven specimens from the ears of the type host, 8. viii. 30. Two specimens from *Rattus edwardsi ciliatus* (Bonhote) taken at Fraser's Hill (4500 ft.), Pahang (Dr A. K. Cosgrave coll. 25. iv. 30), are larger (length 0·43 mm.; breadth 0·34 mm.) and have the scutal and body setae longer, but are otherwise indistinguishable.

Gahrliepia cetrata n.sp. (Fig. 12).

Body oval; striations weak, following contour of scutum; very slightly crenulated; appendages highly sclerotised; pitting on epistome, hypostome, scutum and legs; colour, yellowish white. *Fangs of chelicerae* stout, strongly curved ventrally, dorso-apical tooth prominent, blunt, ventral tooth minute,

arising from a ridge, directed backwards; maxillary setae plain. *Palpi* angulated, with a small tubercle at point of angulation; first palpal seta with a few barbs; second palpal seta plain, rarely with a few barbs, long; on fourth segment three setae, the dorsal long and fine, the lateral plain, the ventral plain or with a few barbs, short; appendiculum with two stout, plumose setae and two pectinate, the spur rather long; palpal claw trifurcate, slender, the lower accessory prong shorter than the upper. *Scutum* longer than broad (mean *L* 189μ; *W* 95μ; pseudostigmata 49μ apart), tongue-shaped, slightly

Fig. 12. *Gahrliepia cetrata* n.sp.

constricted just anterior to fourth pair of scutal setae; anterior margin straight, extremity rounded; scutal setae eight in number, anterior pair the shortest, situated at anterior corners; a pair, longer than the others, on the margins of scutum posterior to pseudostigmata, the two pairs posterior to these placed well within lateral margins; the anterior two pairs coarsely, but sparsely pilose, the others less so. Pseudostigmata on mid-line between the two anterior pairs of setae; *pseudostigmatic organs* capitate, spindle-shaped, the stem short, the head covered with prominent setules, especially towards the base. *Eyes* just visible near margins of scutum, the anterior slightly larger and level with pseudostigmata. *Body setae* eighty to ninety-two in number;

on dorsum thirty to thirty-two, stout, pilose, arranged 1 : 2 : 2 on each side of scutum, then in rows across body approximately as follows: 6 : 6 : 4 : 4 : 2; on venter shorter and finer, pilose to serrate or pectinate, longer posteriorly than anteriorly. *Legs* I and II with terminal segments abruptly narrowed; mean *L*, fore-leg, 212 μ; leg setae plain, serrate, or with a few barbs, a few pectinate on terminal segments; coxal setae single, fine, serrate to pectinate. *Length* (partially engorged) 0·40–0·49 mm.; *width* 0·27–0·33 mm.

Type host: *Rattus edwardsi ciliatus* (Bonhote). Locality: Fraser's Hill (4500 ft.), Pahang, Federated Malay States. *Type* and two paratypes in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Thirteen specimens from the ears and around the anus of the type host, which is only found in the hills (Dr A. K. Cosgrave coll. 25. iv. 30). This species is apparently related to *G. nanus* Oudemans 1910 *b*, but differs in having the palpal claw trifurcate instead of bifurcate and in other details.

Gahrliepia rustica n.sp. (Figs. 13 and 14 *a*).

Body ovoid; striations very faint, following contour of scutum, barely crenulated; pitting inconspicuous, on scutum, epistome, hypostome and legs; appendages strongly sclerotised; colour, yellowish white. *Fangs of chelicerae* stout, strongly curved below, dorso-apical tooth very prominent, sharp, directed backwards, ventral tooth arising from a ridge, prominent, directed backwards; maxillary setae plain. *Palpi* angulated; first palpal seta plain, second palpal seta plain, or with one or two barbs; on fourth segment three plain setae, the dorsal one long; appendiculum with four pectinate setae, one of which is stout, the spur rather long; palpal claw trifurcate, thin, the lower accessory prong slightly shorter than the upper. *Scutum* longer than broad (mean *L* 69 μ; *W* 51 μ; pseudostigmata 30 μ apart) anterior margin very slightly concave; produced behind posterior lateral setae in the form of a bluntly pointed angle, as long again as the distance between the anterior and posterior setae; scutal setae four in number, stout, coarsely pilose to pectinate, anterior pair on the corners, posterior pair on the margins below pseudostigmata; pseudostigmata on, or slightly in advance of, mid-line between scutal setae. *Pseudostigmatic organs* capitate, spindle-shaped, sharply pointed, covered with prominent, long setules. *Ocular shield* and eyes invisible. *Body setae* fine, coarsely pilose to pectinate, ninety to ninety-eight in number; on dorsum thirty-eight to forty, arranged somewhat irregularly, on venter shorter, longer posteriorly than anteriorly. *Legs* I and II with terminal segments abruptly narrowed; mean *L*, fore-leg, 143 μ; leg setae serrate or pectinate, coxal setae single, pectinate. *Length* (partially engorged) 0·32–0·36 mm.; *width* 0·19–0·24 mm.

Type host: *Rattus surifer surifer* (Miller). Locality: Sungei Buloh, Selangor,

Federated Malay States. *Type* and paratype in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Seven specimens from the ears of the type host, 23. vii. 30.

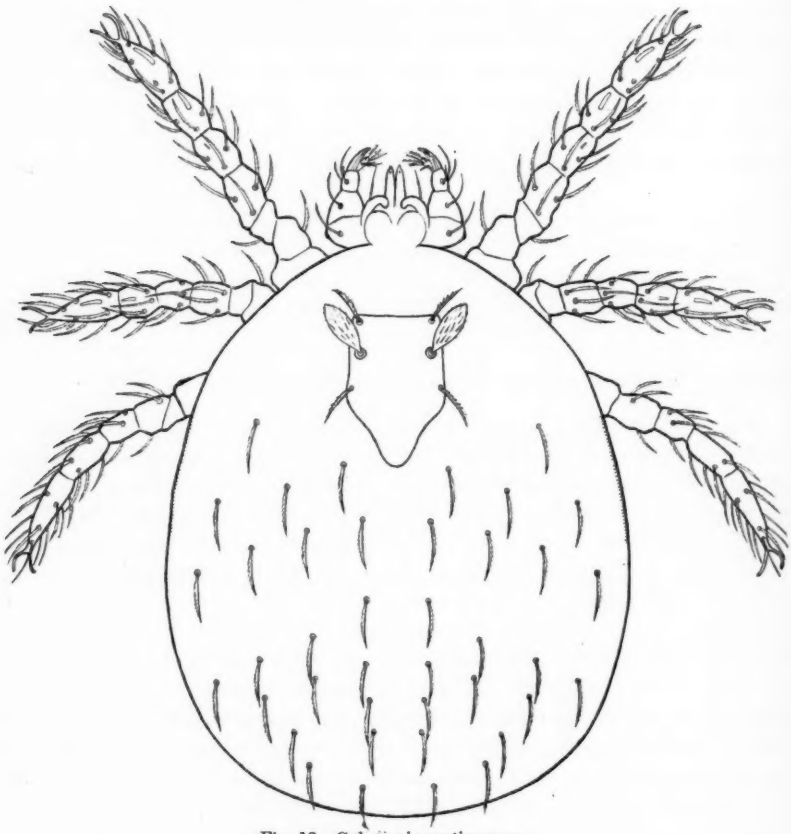

Fig. 13. *Gahrliepia rustica* n.sp.

Gahrliepia turmalis n.sp. (Fig. 14 *b*).

Similar to *Gahrliepia rustica*, but differs as follows: fangs of chelicerae with dorso-apical tooth blunt, not directed backwards, ventral tooth smaller. Palpi markedly angulated with a tubercle at the angle; second palpal seta plain. Setae on scutum and body longer, finer, serrate to weakly pectinate only; on dorsum forty to forty-four; on venter forty-eight to sixty, longer posteriorly than anteriorly. Pseudostigmatic organs missing in all specimens and may prove to differ from those of *G. rustica*. The setae on the hypostome

are plain or serrate instead of plumose as in most other species. *Length* (partially engorged) 0·35–0·41 mm.; *width* 0·22–0·26 mm. Scutum, mean *L* 77 μ; *W* 56 μ; pseudostigmata 34 μ apart. Fore-leg, mean *L* 167 μ.

Type host: *Rattus sabanus vociferans* (Miller). Locality: Sungei Buloh, Selangor, Federated Malay States. *Type* and paratype in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Six specimens from around the anus of the type host, 23. vii. 30.

Genus **Walchia** Ewing 1931.

Walchia enode n.sp.

Body oval, usually with a constriction posterior to third pair of legs; striations very weak, absent over most of body, crenulated only at constriction; faint pitting on scutum, epistome, hypostome and legs.

Fangs of chelicerae stout, short, dorso-apical tooth small, blunt, directed slightly forward, ventral tooth small, arising from a ridge, backwardly directed; maxillary setae plain. *Palpi* small, first palpal seta plain, second palpal seta

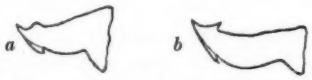

Fig. 14. Fangs of chelicerae. *a*, *Gahrliepia rustica* n.sp.; *b*, *Gahrliepia turmalis* n.sp.

long, plain; on fourth segment three plain setae, the dorsal one long; appendiculum with four strongly pectinate setae; palpal claw slender, apparently trifurcate, accessory prongs unequal, the lower shorter than upper, both very fine and closely adpressed to the main element. *Scutum* longer than broad (mean *L* 63 μ; *W* 53 μ; pseudostigmata 24 μ apart) anterior margin slightly concave, posterior margin produced to form an acute angle; scutal setae four in number, strongly pectinate or plumose, anterior pair on corners, posterior pair on lateral margins at the point where they converge to form the posterior angle, posterior pair slightly the longer. Pseudostigmata below mid-line between setae; *pseudostigmatic organs* capitate, the head balloon-shaped, nearly globular, covered with prominent setules, the stem thin, short. *Ocular shield* and eyes apparently absent. *Body setae* strongly pectinate to plumose, eighty-two to ninety-two in number, variable; on dorsum thirty-two to thirty-eight, arranged approximately in rows as follows, but variable: 8 : 6 : 6 : 6 : 4 : 4 : 2; on venter longer posteriorly than anteriorly. *Legs* with terminal segments tapering abruptly; mean *L*, fore-leg (ten specimens), 152 μ; leg setae serrate or pectinate; tarsal claws unequal; coxal setae single on I and II, four on each of III, strongly pectinate, those on II the smallest. In one specimen there are only three setae on one coxa and in one there are five on one coxa. *Length* (partially engorged) 0·35–0·51 mm.; *width* 0·25–0·38 mm.

Type host: *Rattus mülleri validus* (Miller). Locality: Sungei Buloh, Selangor, Federated Malay States. *Type* and paratype in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Nine specimens from the ears of the type host, 24. iv. 30 and 8. viii. 30. This species is very similar to *W. glabrum* (Walch) 1927, but apart from the difference in the number of coxal setae, the tarsal claws, although unequal, do not exhibit the extreme disparity in size seen in that species.

Walchia lewthwaitei n.sp. (Figs. 15 and 16).

Body generally with a strongly marked constriction posterior to the hind legs; striations very faint, absent except near constriction and scutum, crenulated only at constriction; pitting practically absent. *Fangs of chelicerae* very short, stout, very strongly curved ventrally; dorso-apical tooth prominent, blunt, tending to point forwards, ventral tooth small, arising from a ridge; maxillary setae plain. *Palpi* very small; first palpal seta plain, second palpal seta plain, long; on fourth segment three plain setae; appendiculum with four strongly pectinate setae; palpal claw trifurcate, very slender, lower accessory shorter than upper, both closely adpressed to main element. *Scutum* very weak, practically invisible in mounted specimens, as broad, or broader, than long (approximate mean *L* 37 μ; *W* 39 μ; pseudostigmata 18 μ apart) more highly sclerotised in centre than elsewhere, anterior margin slightly concave, posterior margin produced in a somewhat irregular line; scutal setae four in number, very short, strongly pectinate or plumose. Pseudostigmata on midline between setae; *pseudostigmatic organs* capitate, the head balloon-shaped, almost globular, covered with prominent setules; the stem thin, short. *Ocular shield* and eyes apparently absent. *Body setae* variable in number, eighty-two to ninety-four; on dorsum thirty-four to forty, very short, fine, strongly pectinate or plumose, arranged in rows approximately as follows: 8 : 6 : 6 : 6 : 6 : 2 : 2; on venter short, very fine, strongly pectinate or plumose, longer posteriorly than anteriorly (not shown in figure). *Legs* very short, the terminal segments tapering abruptly; mean *L*, fore-leg, 98 μ; leg setae serrate to strongly pectinate, tarsal claws unequal; coxal setae single on I and II, six on each of III; on coxa II very small, plain, serrate, or with a few barbs, other coxal setae strongly pectinate or plumose. *Length* (partially engorged) 0·32–0·46 mm.; *width* 0·24–0·33 mm.

Type host: *Rattus rattus* (probably *diardi* Jentink). Locality: Kuala Lumpur, Selangor, Federated Malay States. *Type* and paratype in British Museum; paratypes in United States National Museum, Molteno Institute, Cambridge, and King Edward VII College of Medicine, Singapore.

Described from nine specimens collected by Dr R. Lewthwaite (after whom the species is named) while investigating tropical typhus in August, 1927. Also found on *Rattus surifer surifer* (Miller), Sungei Buloh, Selangor, 21. vii. 30 and on *Sciuropterus* (*Hylopetes*) *belone* Thos., Sungei Buloh, Selangor, 24. vi. 30.

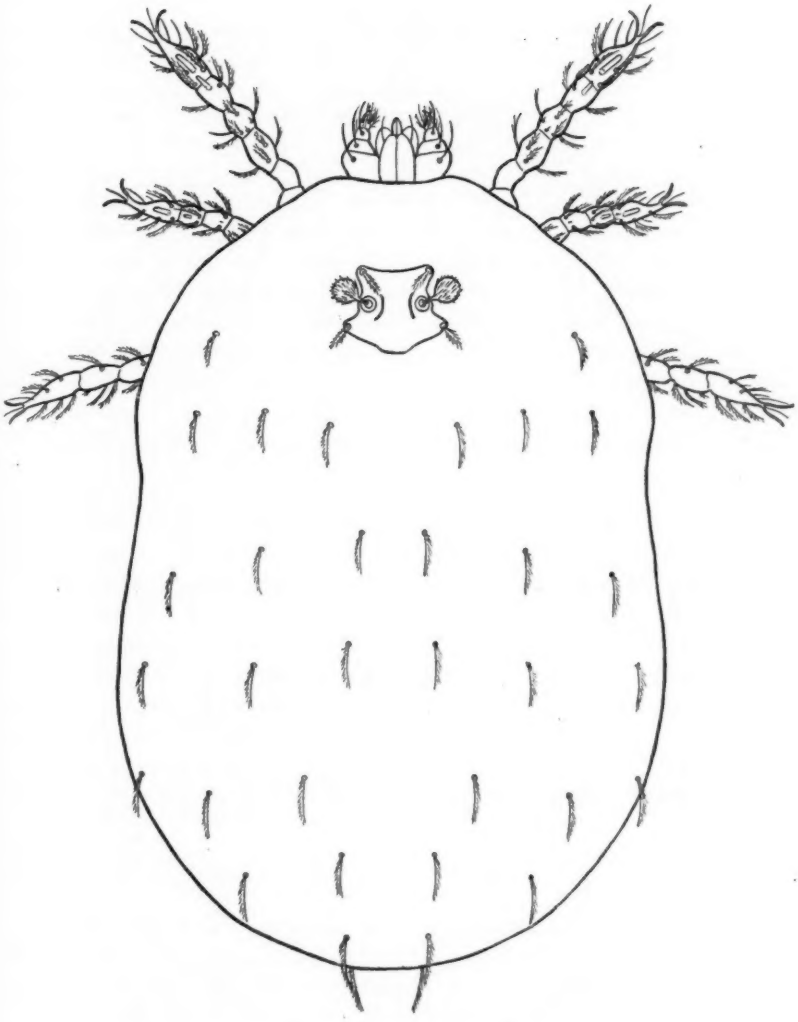

Fig. 15. *Walchia lewthwaitei* n.sp. Dorsal aspect.

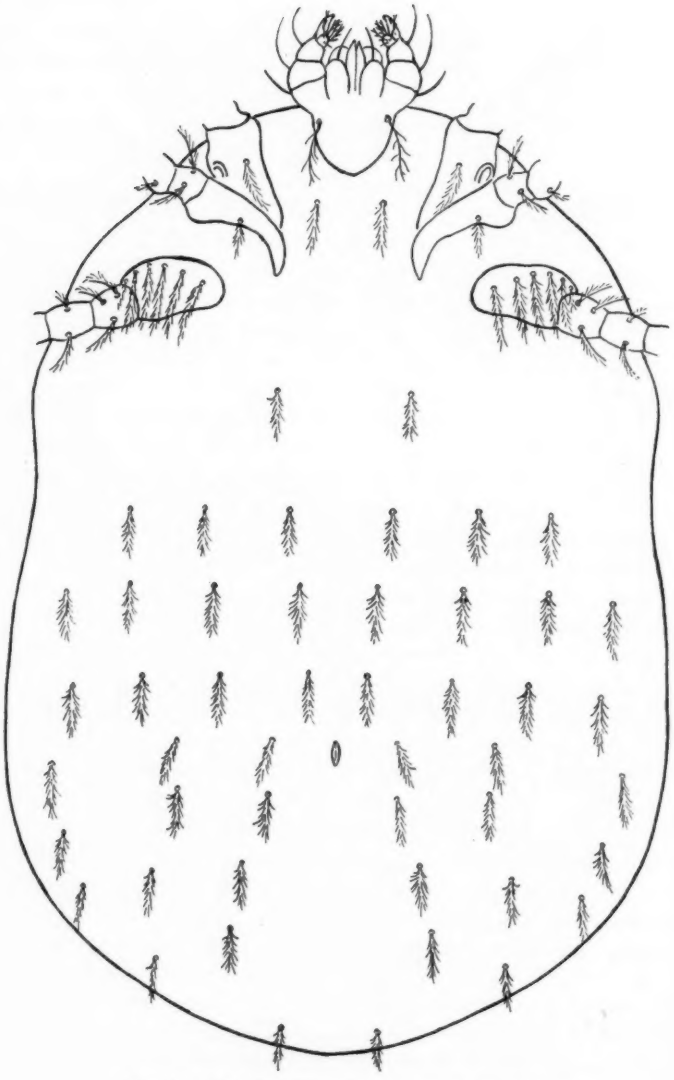

Fig. 16. *Walchia lewthwaitei* n.sp. Ventral aspect.

Walchia pingue n.sp.

Body oval, with a constriction posterior to third pair of legs; striations very weak, absent over most of body, crenulated only near constriction; very faint pitting on scutum, epistome and legs; colour, greenish white. *Fangs of chelicerae* short, stout, dorso-apical tooth small, blunt, ventral tooth small, arising from a ridge, directed backwards; maxillary setae plain. *Palpi* small; first palpal seta plain, second palpal seta plain, long; on fourth segment three plain setae, the dorsal one long; appendiculum with four strongly pectinate setae; palpal claw slender, apparently trifurcate, but impossible to distinguish with certainty the exact number of prongs in specimens. *Scutum* very weak, longer than broad (approximate mean *L* 57 μ; *W* 47 μ; pseudostigmata 21 μ apart) anterior margin concave, posterior portion produced to form an acute angle; scutal setae four in number, anterior pair at corners of scutum, posterior pair at base of angular projection, short, strongly pectinate or plumose, the posterior pair the longer. Pseudostigmata below mid-line between scutal setae; *pseudostigmatic organs* capitate, the head balloon-shaped, almost globular, covered with prominent setules, the stem short and thin. *Ocular shield* and eyes apparently absent. *Body setae* seventy-six to eighty-six in number; on dorsum thirty-two to thirty-four, strongly pectinate, arranged in rows approximately as follows: 8 : 6 : 2 : 6 : 4 : 4 : 2; on venter smaller, strongly pectinate, longer posteriorly than anteriorly. *Legs* with terminal segments tapering abruptly, mean *L*, fore-leg, 162 μ; leg setae serrate, pectinate or plumose; tarsal claws unequal; coxal seta single on I and II, three on each of III; on coxa II small, plain or with a few barbs, other coxal setae strongly pectinate or plumose. *Length* (partially engorged) 0·48 mm.; *width* 0·33 mm.

Type host: *Rattus edwardsi ciliatus* (Bonhote). Locality: Fraser's Hill (4500 ft.), Pahang, Federated Malay States. *Type* in British Museum; paratype in King Edward VII College of Medicine, Singapore.

Two specimens from the ear (Dr A. K. Cosgrave coll. 25. iv. 30). This species is very similar to *W. glabrum* (Walch) but the tarsal claws, although unequal, are much more developed.

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SOME TICK INVESTIGATIONS IN KENYA COLONY.

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Kenya Colony offers a rich field for the study of ticks and tick-borne diseases. The natural conditions under which both ticks and the diseases exist range from those of the hot, low-lying coastal lands to the equable climate of the highlands culminating in the colder upland country and mountain slopes. The variety of conditions is also attributable to the extensive tracts of bush-covered country, the high plateaux with their scanty growth of vegetation, and to the forest-clad ridges and mountains.

The climate is peculiar: the rainfall varies a good deal from district to district; and exposure to the direct heat of the sun and the desiccating power of the winds is moderated by the influence of forests, bush, and dense growth of long grass.

Ticks are abundant and there is a fairly large diversity of species. One has to walk but a short distance into the well-known Athi Plains, thickly covered with long grass and with its scattered thorn trees, to become acquainted with myriads of minute "pepper ticks"—the larvae of *Rhipicephalus pulchellus*—which readily adhere to one's person. A journey through the dense scrub in the Masai Reserve will reveal a variety of species crawling on one's clothing; and even a cursory examination of one's dog, or the cattle in most parts of the Colony, will disclose a wealth of ticks in all stages of development.

Game, vermin and other wild fauna contribute a variety of species of ticks, some of which are common to domestic stock, while others appear to possess a predilection for some specific host or a very limited number of hosts.

R. appendiculatus is a fairly general feeder; it infests the ears of cattle and is often found on the body generally; it attaches itself to sheep and goats, and, in fact, to all domestic animals. It has been collected frequently from buffalo (*Syncerus caffer*), waterbuck (*Cobus ellipsiprymnus*), bushbuck (*Tragelaphus scriptus*), and zebra (*Equus burchelli granti*). *Haemaphysalis leachi* is as abundant on jackal (*Canis aureus*), leopard (*Felis pardus*), and lion (*Felis leo*) as on the domestic dog and cat. *Amblyomma variegatum*, as shown below, has a wide range of hosts. *A. tholloni* has been found, mainly, on the elephant (*Elephas africans*) and *A. marmoreum* is found on the rhinocerus (*Rhinoceros bicornis*) as well as on some Reptilia and Chelonia. *Dermacentor rhinocerotis*, too, favours the rhinoceros, and *Aponomma exornatum* seems to prefer the iguanas.

Rodents, and birds such as spur-fowl, partridge and guinea-fowl, provide suitable hosts for the larval forms of ticks, which in more developed phases prefer the higher mammals.

There is evidence that certain species of ticks in Kenya Colony are restricted in their distribution, but this evidence, in many cases, is merely circumstantial; it refers to particular species, some of which are incriminated in the transmission of diseases to stock, and it is based on the prevalence of those diseases in isolated areas, and their absence in other places.

If a known tick-borne disease is absent from a locality where there are susceptible animals, it can only be deduced that the causal organism is absent; the vector of that organism may be abundant. On the other hand, the existence of a particular tick-borne disease is no proof of the presence of any one species of tick, for it is known that two or more species are capable of transmitting the same disease.

Biliary fever in dogs may point to the infestation of that animal by infective *Rhipicephalus sanguineus* or *Haemaphysalis leachi*. An outbreak of East Coast fever may result from the "bites" of infected *R. appendiculatus*, *R. evertsi*, *R. simus* or *R. capensis*. Redwater may be transmitted by *Boophilus decoloratus*, *R. bursa* or *R. evertsi*. *Amblyomma hebraeum* may be responsible for the transmission of heartwater, or *A. variegatum* may be the vector of the disease. Indeed, so little is yet definitely known of the tick species in Kenya Colony and their potentialities for conveying pathogenic organisms, that the distribution and the economic importance must be studied by collecting specimens in the field and making exhaustive observations of the conditions under which they live and thrive, and by supplementing this information with careful experiments in the laboratory on the biology of the species and their capability of carrying organisms pathogenic to man or animal.

The Colony is divided into "clean" and "dirty" areas, where "clean" indicates the comparative absence of East Coast fever, and "dirty" signifies that the disease is endemic. Within the "clean" alienated areas there are patches of land where *R. appendiculatus*, the chief vector of the disease, is common; within the endemic alienated areas there are small localities where the chief vector is entirely absent. Our knowledge of the Native Reserves is more or less meagre.

An enormous amount of field and of laboratory work is necessary yet to direct further research along avenues which may lead to proper control and complete eradication of ticks and tick-borne diseases.

Some results of tick investigations in the field in Kenya Colony have been published (Lewis, 1931).

It is thought desirable to publish the results of preliminary observations and studies on the life history of some species commonly found in Kenya Colony so that the information may be utilised in experimental transmission of diseases. Brief notes, also, are given on the distribution of the species in nature.

Rhipicephalus pulchellus (Gerstäcker 1873).

This species is commonly found on the Athi, the Kamati, and the Kapiti Plains, in the Northern Frontier District, and in the Masai Reserve. It is somewhat rare in the majority of alienated areas, except those districts which adjoin the infested areas mentioned above.

Montgomery (1911–12), dealing with East Coast fever transmission experiments, states the "Yellow-backed tick (*R. pulchellus*), abundant on the Athi Plains, could not be fed on cattle or other large animals in its larval state. The tick abounds near, and in, an infected area near the Athi river." In 1913–14 the same author says that "considerable difficulty has been experienced in obtaining any information about the habits of *R. pulchellus*, the common tick of the Athi Plains."

Several attempts have been made to feed the larvae of this tick on calves and sheep with no success. The larvae die within a few days. An endeavour was made to feed them on my hands; this also failed. The local hare (*Lepus capensis crawshayi*) proved a more suitable host for all stages of the tick. The life cycle, completed on the hare, occupies a minimum period of 151 days, distributed as shown below:

	Time required in days	Temperature ° C.
From the time egg is laid to the emergence of larva	36	22–26
Larva hardens	7	—
Larva feeds on host	3	—
Larva drops off host and moults to nymph	12	21–24
Nymph hardens	7	—
Nymph feeds on host	3	—
Nymph drops off host and moults to adult	26	21–26
Adult hardens	7	—
Adult female feeds on host	6	—
Replete female drops off host, begins to lay after ...	8	26–29
From the time egg is laid to the emergence of larva	36	21–26
	Total 151 days	

R. pulchellus is a three-host tick on the hare. Although the life cycle can be completed when using this host, the small percentage of larvae which fed to repletion indicates that the host is not the normal one in nature. The nymphs and adults feed much more readily than the larvae.

The species is distributed throughout the hotter regions of Kenya Colony and appears to find favourable conditions on the game-infested plains at altitudes not above 5000 to 6000 ft.

I have collected specimens from cattle, sheep, goats, pigs, horses and dogs, but they are more often found on zebra, buffalo, wildebeeste, waterbuck and warthog. Specimens have been found on man also.

Rhipicephalus simus Koch 1844.

Lounsbury (1903), writing on ticks and African Coast fever, states of *R. simus* "the adult appears to attach most freely to the legs. The ox may not be an important host of the species. At Nelspruit more than were taken from

all the cattle together were found on a single dog, and the back, neck and flanks of this animal were freely infested." According to Lounsbury (1906) this tick seems to have a very limited distribution in Cape Colony and it is recorded only from a few eastern coastal districts. The ox appears to be unsuitable as a host for the larva, very few of the immense numbers of specimens which we have applied to various parts of the body having fed to repletion. The dog is a much-liked host and all the specimens used in the tests were fed as larvae on this animal. The nymphs and adults do not appear to dislike cattle.

In Kenya Colony the tick is very widely distributed. It is found on the plains, in the bush and forest, and it is an exceptionally common pest on rats and in rats' nests.

In rearing the tick, trials were made to feed them on a calf and sheep, with similar results to those of Lounsbury with the ox. On the hare the larvae did not very readily attach themselves to the ears. About 40 per cent. fed to repletion.

Using the hare as host, the life cycle, as outlined below, occupied a minimum period of 142 days.

	Time required in days	Temperature ° C.
From the time egg is laid to emergence of larva	31	21–27
Larva hardens	7	—
Larva feeds on host	3	—
Larva drops off host and moults to nymph ...	11	23–26
Nymph hardens	7	—
Nymph feeds on host	5	—
Nymph drops off host and moults to adult ...	27	21–25
Adult hardens	7	—
Adult female feeds on host	7	—
Replete female drops off host, begins to lay after	6	23–26
From the time egg is laid to emergence of larva	31	21–27
	Total 142 days	

R. simus is a three-host tick. The hare does not seem to be the normal host for the larval stages. The nymphs and adults feed readily on the hare and, from field observations, on most animals. *R. pulchellus* seems to be similar to *R. simus* in its behaviour. *R. simus* is very frequently found in the nests of rats, particularly those of *Arvicanthus abyssinicus nairobae*. It is also found in the nests of *Rattus coucha panya*, *Otomys abyssinicus elassodon* and *Lemniscomys striatus*.

From the data obtained by examination of a collection of ticks sent to me by Mr J. I. Roberts, Entomologist, of the Medical Research Laboratory, Nairobi, it appears that nymphal *R. simus* feed readily on rats, the larvae also feed fairly readily, but probably to a lesser degree than nymphs; the adults are very rarely collected from the host itself, whereas they abound in nests, even at a depth of two to three feet underground.

It is interesting to note that Veneroni (1928) gives a brief description of a

temporary paralysis in young children in Italian Somaliland, thought to be due to *R. simus* Koch, which is always found in native huts.

R. simus has been collected by me at altitudes ranging from that of the seashore at Tiwi to about 11,000 ft. on Mount Kilimanjaro, where it was obtained from grass.

Other hosts of the nymphs and adults are: jackal, dog, sheep, cattle, hare, porcupine, bush-pig, ant-bear (*O. aethiopicus*), warthog, bushbuck, eland, wildebeeste, buffalo, rhinoceros, zebra, greater kudu, leopard, lion, serval cat and cheetah.

Hyalomma aegyptium (Linnaeus 1758) Koch 1844.

(*Hyalomma impressum albiparmatum* Schulze and Schlottke 1930.)

H. aegyptium is very frequently met with in Kenya Colony. Its hosts are numerous; it has not been inculpated in the transmission of disease to domestic stock, but in one instance there is strong suspicion of its causing paralysis in sheep and lambs in parts of the Rift Valley, Kenya Colony (Lewis, 1931), but experimental evidence that the tick is responsible for the paralysis is lacking.

The life cycle has been worked out in detail by Nuttall (1913) who writes: "*Hyalomma aegyptium* usually requires three hosts upon which to feed in its larval, nymphal and adult stages. When larvae are placed on hedgehogs, however, about half of them drop off as gorged nymphae, so that upon this host, contrary to what occurs when they are put on sheep, they behave like two-host ticks. This peculiar behaviour on hedgehogs is doubtless due merely to the gorged larvae remaining entangled amongst the host's spines.... When they (the ticks) drop off as gorged nymphs from hedgehogs they do so 25–46 days after the larvae are put on the host."

Patton and Cragg (1913) remark: "In a recent paper Nuttall states that the larvae of *Hyalomma aegyptium* will feed on the ram, hedgehog and guinea-pig, and he has raised all stages on these animals; it is doubtful, however, whether these animals are the natural hosts." These two authors agree that Hyalomma species require three hosts to complete the life processes; but add that *H. aegyptium* is peculiar in its habits, as its larvae do not feed on the same species of host as the adults. Patton carried out a long series of experiments with this tick to determine the particular host or hosts it feeds on. As this is also my aim, the following life cycle of *H. aegyptium* in Kenya is of particular interest, for Patton observed in respect to the hosts (cattle, sheep, goat, pig, dog, cat, rabbit, guinea-pig, rat and pigeon) that a few larvae attached themselves to guinea-pigs and rats, but refused to feed on the other animals. He adds: "It is possible that the palm squirrel, *Funambulus palmarum*, and the hare, *Lepus nigricollis*, are the hosts of the larvae; nymphs have been found on the squirrel. It is, however, certain that the larvae do not attach themselves to any of the common domestic animals in Madras. Lounsbury has had very similar experiences in South Africa."

I have carried out a number of experiments with this tick on calves, sheep, chickens and hares.

On all but hare the cycle is similar to that described by Nuttall (1913), a very large percentage of larvae became attached and fed to repletion. On the hare, however, the life cycle requires only two hosts. The results of many rearings are tabulated below, the minimum period being given for each phase. The shortest time to complete the life cycle on the hare is 138 days.

	Time required in days	Temperature ° C.
From the time egg is laid to emergence of larva	38	23–25
Larva hardens	7	—
Larva feeds on host, moults to nymph without dropping off; nymph feeds and drops gorged in	13	—
Nymph drops off host and moults to adult ...	17	24–25
Adult hardens	7	—
Adult female feeds on host	5	—
Adult female drops off host, begins to lay after	13	24
From the time egg is laid to emergence of larva	38	22–25
	Total 138 days	

The larvae moulted to nymphs, on the host, on the sixth day after attachment; engorged nymphs dropped off the host on the seventh day after moulting. An extremely small percentage of gorged larvae dropped off the host to moult.

After moulting from the larval stage some of the nymphs crawled about the ears before attaching themselves, whereas others became attached immediately at the original point of fixation of the larva.

There is hardly any doubt that the hare (*Lepus capensis crawshayi*) is one of the natural hosts for the earlier stages of *H. aegyptium*.

Adult specimens have been collected in the Colony from cattle, sheep, goat, ostrich, camel, oryx, roan, zebra, buffalo, rhinoceros, eland, Grant's gazelle, warthog and impala. Nymphs have been collected from hare, and larvae and nymphs from dog. The species has been found at altitudes ranging from sea-level to 8500 ft.

Haemaphysalis aciculifer Warburton 1913.

This species was taken from *Cobus thomasi* (an antelope) on the north-east shore of Lake Edward, Uganda. I collected many specimens of nymphs and adults from bushbuck, buffalo, waterbuck, duiker, warthog and hare in a limited stretch of land in the Karati Forest, near Naivasha. Only once has it been taken from a domestic animal, a goat in the Somali village between the Karati Forest and Naivasha township. It usually occurs at about 7500 ft. The minimum time occupied to complete the life cycle on the hare is 127 days, distributed as follows:

	Time required in days	Temperature ° C.
From the time egg is laid to emergence of larva	20	22–25
Larva hardens	7	—
Larva feeds on host	3	—
Larva drops off host and moults to nymph ...	22	21–24
Nymph hardens	7	—
Nymph feeds on host	3	—
Nymph drops off host and moults to adult ...	22	21–25
Adult hardens	7	—
Adult female feeds on host	7	—
Adult female drops off host and begins to lay after	9	19–21
From the time egg is laid to emergence of larva	20	23–25
	Total 127 days	

H. aciculifer is a three-host tick on the hare. In one experiment a nymph became fully fed and dropped off the host in 2 days.

Amblyomma variegatum (Fabricius 1794).

This is a common species in Kenya Colony; it has been proved to transmit heartwater in the adult stage (Daubney, 1930). Owing to the difficulty of rearing this tick, further experiments in transmission of the disease have been difficult to carry out. Nuttall (1915) states: "In the case of *Amblyomma variegatum* we can only record a partial life history as our attempts to raise the species were not wholly successful; we, moreover, lost the strain." "Attempts to raise the larvae failed. The life history appears to be essentially similar to that of *A. hebraeum*."

After many attempts to rear this species, I succeeded in elucidating the complete life cycle which is outlined below. The shortest period taken at each phase is given:

	Time required in days	Temperature ° C.
From the time egg is laid to emergence of larva	86	19–26
Larva hardens	7	—
Larva feeds on host	7	—
Larva drops off host and moults to nymph ...	22	25–27
Nymph hardens	7	—
Nymph feeds on host	7	—
Nymph drops off host and moults to adult ...	24	24–27
Adult hardens	7	—
Adult female feeds on host	10	—
Adult female drops off host and begins to lay after	18	24–27
From the time egg is laid to emergence of larva	86	19–26
	Total 281 days	

A. variegatum, in the above cases, was fed on the hare, chicken and sheep. Like *A. hebraeum*, it is a three-host tick on these animals. The species is widely distributed in the Colony and has been collected at altitudes ranging from sea-level at Mombasa to about 8500 ft. in the highlands of the Uasin Gishu district.

Its hosts are: cattle, sheep, goat, eland, roan, kongoni, steinbuck, zebra, rhinoceros and ant-bear. Larvae have been collected from the necks of spur-fowl in the arid districts of the Northern Frontier Province. Nymphs and adults are commonly found on domestic stock.

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THE OVA OF *AËDES* (*FINLAYA*) *GENICULATUS* OLIVIER

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The British species of *Aëdes*, *A.* (*Finlaya*) *geniculatus*, lays its eggs on the damp surfaces of tree-holes containing water; or, if the expanse of water in the hole be large, often directly upon the water surface.

There are at least two generations annually: one appearing in midsummer from the individuals which have emerged in the months of spring, and the other occurring in early to late autumn from the members of the summer generation. "At least two generations," since it is impossible to say whether the female under natural conditions lays successively more than one batch of eggs. If the blood supply is abundant, it seems highly probable from the results of laboratory experiments that successive egg-laying does take place. Factors inimical to the species in its natural environment would, however, seem to be great, for, although there are often innumerable tree-holes in our woods, large numbers of adults are rarely, if ever, seen. Occasionally the larvae are very numerous in certain tree-holes, and most tree-holes, especially those in beech trees in summer, may be relied upon to contain at least a few larvae. In winter only a few holes harbour larvae. While it is true that some of the ova of *Aëdes geniculatus* exhibit the phenomenon of delayed hatching, even up to as late as the months of February and March in the following year, nevertheless, as our records have shown, the number of larvae present during the winter is never as great as in summer. The inimical factors would consequently appear to act either against oviposition during the autumn or else against the earliest stages of the larval development.

During the cold winter months the larvae are far more torpid than the overwintering larvae of the other British mosquitoes, yet large numbers of eggs laid in autumn habitually hatch in December or somewhat later. Despite this fact, laboratory experiments have proved that neither the larvae (at any stage) nor the ova can withstand freezing, even when the temperature of the surrounding ice is maintained at 30–32° F.

In August 1931 adults were caught in the Wisley woods in connection with bird malaria investigations. Females were fed on both human and avian blood, and 4 days later viable ova were laid. The average number of ova deposited by each female was 46. The eggs were laid singly, and were arranged

irregularly over the surface of half-submerged beech leaves or on the open water surface in a small Petri dish. In appearance the ova were very similar to those of *Aëdes* (*Stegomyia*) *aegypti*, but could be readily distinguished from the latter by their greater girth. The following are the relative and comparative measurements of the ova of both species.

	Aëdes aegypti		*Aëdes geniculatus*	
Average	Length	0·654 mm.	Length	0·654 mm.
	Breadth	0·163 mm.	Breadth	0·215 mm.
	Number	73 per batch	Number	46 per batch

From these ova healthy larvae were obtained which were easily reared in the laboratory to the adult stage. Adult larvae and pupae were obtained from the tree-holes also in August, and the emergent adults were confined in large insect cages. Under such conditions fertilisation of the females did not, however, occur, although large numbers of males were associated with the females in the cages.

Some of the females which had been kept with males for 7 days were fed on human blood. After the second blood meal a few of the mosquitoes developed ova and oviposition took place 4 days later. In appearance and size these ova were exactly similar to those of the wild females, but the average number of eggs laid in this case per female was only 14. None of these eggs hatched, and microscopical examination proved that all were infertile.

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THE NASAL BOT FLY, *CEPHENOMYIA AURIBARBIS* MEIGEN (DIPTERA, TACHINIDAE) OF THE RED DEER, *CERVUS ELAPHUS* L.

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(With 3 Figures in the Text.)

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I. Tachino-oestrid parasites of the red deer in Britain.

The red deer (*Cervus elaphus* L.) in Europe is the host of four tachino-oestrid species belonging to three genera, *Cephenomyia auribarbis* Mg., *Pharyngomyia picta* Mg., *Hypoderma diana* Br., and *Hypoderma actaeon* Br. Of these the larvae of the first two are found in the naso-pharynx, whilst the larvae of the two last are subcutaneous parasites, which issue at maturity from "warbles" on the back of the host like *H. bovis* and *H. lineatum* of cattle. *H. actaeon* has not been recorded in Britain, and whilst Austen (1898) states that Curtis recorded *P. picta* from the New Forest in 1823 and that Bracy Clarke mentioned the larva of this species in 1847, there is curiously no further record of its occurrence in Britain. So far as *C. auribarbis* and *H. diana* are concerned, their distribution in Britain, as judged by the collections of the Natural History Museum, London, and the Royal Scottish Museum, Edinburgh, appears to be co-extensive with that of the deer forests of Scotland, over which the host ranges widely.

Adults of *C. auribarbis* have been captured in the counties of Sutherland, Ross and Cromarty, Inverness, Aberdeen and Perth. Bloomfield (1898) states how larvae were collected from the "heads" of red deer killed in Strathdon and other places of Aberdeenshire. In one deer thirty larvae, each nearly 1 in. in length, were found near the root of the tongue. The specimens were identified by Austen (1898) as probably belonging to *C. auribarbis*. It is not unlikely that *C. auribarbis* occurs more commonly than is supposed and that

the larvae hidden away in the naso-pharynx are apt to be overlooked by ghillies and stalkers, when the carcass of the deer is dressed. Again, the growth of the larvae is very slow, so that the season for deer shooting is over before they have attained a size that would make them readily discernible to the casual observer, and only then, if the throat were carefully examined.

The genus Cephenomyia is represented in Europe by three species other than *C. auribarbis*. Of these, *C. nasalis* L., parasite of the reindeer, occurs in Lapland; *C. stimulator* Clark occurs with its host the roe deer in Germany and Austria, and *C. ulrichi* Br., which parasitises the elk, is distributed over central Europe, Lithuania and Finland. Although the roe deer is found in Britain, there is no record of its parasite here. It is thus evident that of the naso-pharyngeal tachino-oestrids of the Cervidae, Britain has but two species, *C. auribarbis* and *P. picta*, of which the latter has not been encountered for more than a century. The larvae of the two markedly resemble each other. *C. auribarbis* may be distinguished by the close approximation of the bases of the antennae, which are widely separate in *P. picta*. In the former there is no anterior transverse band on the dorsal aspect of the tenth body segment, and, because of the greater obliquity of the posterior stigmata, their ventral horns are most divergent.

II. Description of *C. auribarbis*.

The genus Cephenomyia may be briefly described as follows:

Robust flies, body glistening black, densely clothed with hairs both black and of lighter hue. Head broader than the thorax. Facial depression cordate; below, continued into a narrow groove; above, between the bases of the antennae divided into two by a slight ridge. Antennae short and thick, third segment largest, bulbous. Arista thickened at the base, naked. Haustellum short. Palps clavate, hairy at the extremities. Wing membrane slightly wrinkled, smoky. First posterior cell open, but narrowed by the abrupt up-curving of the fourth longitudinal vein starting from an elbow beyond the posterior cross-vein; straight part of vein continued beyond the elbow for a brief course. Abdomen hemispherical. Terminal abdominal segment large and rounded. Ovipositor not exserted in female.

The accompanying sketch of the female of *C. auribarbis* (Fig. 1) was made from a specimen captured at Corrour, Invernessshire, by W. Eagle Clark in 1918 at an elevation of 1000 ft. It was in the collection of Mr P. Grimshaw, Royal Scottish Museum, to whom I am indebted for the gift of the specimen.

Cephenomyia auribarbis Mg. is densely pubescent, black, about $\frac{5}{8}$ in. long. Head has silvery reflections on frons, cheeks and facial pit; vertex, frons and cheeks with black hairs; lower part of face with a thick tawny beard obscuring the atrophied mouth-parts. Antenna dark reddish brown, with brown arista. Mesonotum with five indistinct naked lines, which scarcely interrupt the otherwise continuous pubescence occurring in three bands of colour, an olive-brown pre-sutural similar in colour to the hair on the posterior region of the head, a black post-sutural and a yellowish grey scutellar. Sides of thorax invested with light tawny hairs, with a dense tuft of yellowish white hairs anterior to base of wings. Base of abdomen (segments I and II) invested with dirty yellow hairs, third and fourth with black and a posterior fringe of yellow hairs; terminal segment with greyish white hairs. Sternites of

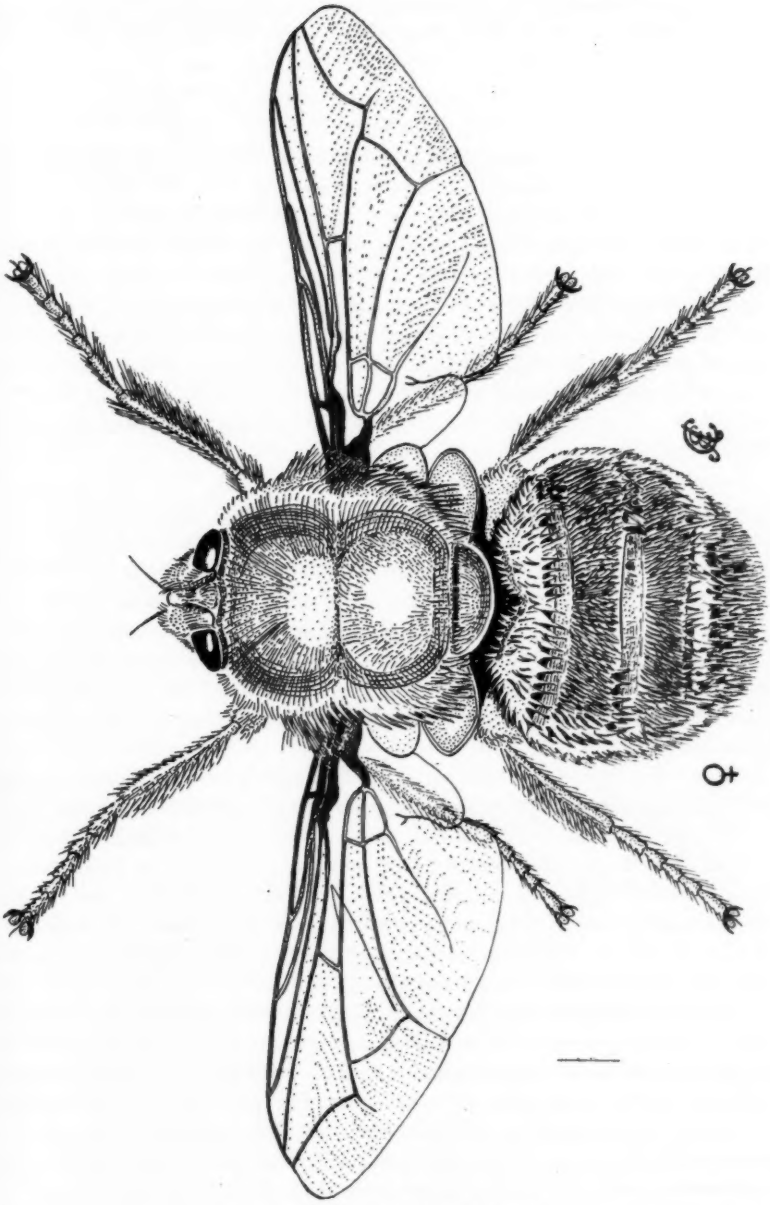

Fig. 1. *Cephenomyia auribarbis* ♀. ×5.

abdomen clothed with long white silky hairs. Legs black, femora with dense grey and black hairs, tarsi reddish brown with apices of segments darker. Wing 12 mm. long, membrane greyish, veins brownish black, clouded with brown. Squamae greyish white, edged with brown. Halteres dark brown with white apices.

III. Notes on the life history and habits of Cephenomyia.

The adult female Cephenomyia is larviparous. It hovers in flight near the head of its host and darts intermittently at its nose. At each attack it ejects a drop of fluid containing one or more larvae into the nostrils of the deer. Altogether each female deposits 500–600 larvae. According to Brauer (1863) and Bergman (1917) the host does not submit quietly to attack, but snorts repeatedly, stamps its feet and behaves generally as if trying to defend itself against an enemy that persists in annoying it. By means of their strongly reflexed mouth-hooks the first-stage larvae attach themselves securely to the mucous membranes of the nose. The resulting irritation induces sneezing, which is often accompanied by a catarrhal condition and extravasation of blood.

Larvae of the first instar measure 1–3 mm. in length. They occur normally in the nasal cavities of the host from July to February. Larvae of the second instar migrate to the pharynx, where, according to Brauer (*loc. cit.*) they increase in length up to 15–20 mm. and are to be found from February to May. After a second ecdysis, the third instar appears and continues to increase in length up to 40 mm., when it becomes full grown in May or June. Larvae of this instar occur in groups of about 30–60 in the pharyngeal recesses and occasionally in the frontal sinuses, whence, at maturity they are ejected by the violent sneezing of the host. Pupation, which occupies 20–30 days, occurs in the soil.

The time occupied by the larva in development is 10–11 months, of which 8 months are said to be spent as the first instar. This remarkable prolongation of the first stadium, which is also characteristic of other tachino-oestrids, such as Hypoderma and Oestrus, has been noted by various authors, who have, however, advanced no satisfactory explanation of its occurrence. In the case of nasal tachino-oestrids, retardation of development is apparently correlated with the continued residence of the first-instar larvae in the nasal cavities. Hadwen (1926) suggested that it may be due to the subjection of the larvae to the prevailing low temperatures of the inspired air during the winter months. In answer to this, it must be remarked that the inspired air is warmed to body temperature in passing over the turbinate folds before reaching the larvae in the upper part of the nasal cavities. Certainly the prolongation of the first larval stadium of Hypoderma cannot be attributed to low temperatures in that, from the time of penetrating the skin of the host, they are continuously subject to body temperatures that are more or less constant.

Fatalities in host animals are frequently ascribed to the activities of the larvae of different species of Cephenomyia. Their presence in the pharynx is

accompanied by a local inflammation. According to Bergman (1917) the tunica propria is infiltrated with cells and becomes thickened. Hyperaemia and bleeding occurs in the region of the recess. Natvig (1916) noted as many as 130 larvae of *C. nasalis* in the throat of a single reindeer and he attributed death to suffocation caused by larvae entering the larynx. This opinion is supported by Bergman (*loc. cit.*), who states that the larvae frequently pass into the lungs and cause death from pneumonia. An elk, from which Bau (1920) obtained 20 larvae of *C. ulrichi*, showed considerable signs of distress and weakness. It seemed scarcely possible that the extreme debility of the host could have been due to the small number of parasites present. Apparently at a loss to find a competent explanation, he suggested (p. 542) that some of the larvae may have penetrated to the brain. That the larvae show considerable powers of burrowing is shown by his discovery of a mature larva, which had bored into the wall of the naso-pharynx for more than half its length. We have no reason to disbelieve this fact, but it seems scarcely credible that the larvae could penetrate the bony cranium to reach the brain. Again, the author does not state whether he recovered any specimens of the larvae from the brain.

Recently Walker (1929) has attributed the mortality of large numbers of deer in Colorado in the spring to a species of Cephenomyia. In one animal, where 54 larvae were counted in the naso-pharynx, the tissues of the lower nasal passages were highly inflamed. The upper left nasal sinuses were observed to be infected, and the infection was said to have spread to the left cerebral lobe of the brain, where a haemorrhage was noted. Amongst the reindeer in Lapland a similar condition was noted by Bergman (1917, p. 58). In the spring the affected animals suffer from giddiness, turn in circles, stagger, fall down and finally succumb. The only pathological changes observed were hyperaemia of the brain and cerebral oedema, said to be the result of severe inflammation caused by the presence of a large number of Cephenomyia larvae in the pharynx and nostrils.

IV. The early third-stage larva of *C. auribarbis*.

On 23. i. 1931 a stag of the red deer, which had been killed at Blair Atholl, Perthshire, was carefully examined shortly after death by W. C. Miller, M.R.C.V.S., Department of Animal Genetics, Edinburgh University. Amongst the fat in the pelvic region a small cyst-like swelling was observed, lying just mesial to the right common iliac vein and close to the mesorectum. It was found to contain a living larva, which was handed to me for identification. It was recognised as belonging to the genus Cephenomyia, and a comparison of the specimen with the description given by Brauer (1863, p. 197) showed it to be an early third-stage larva of *C. auribarbis*. Its abnormal occurrence in the pelvic region can only be explained by supposing that it may have been swallowed, after which it had burrowed through the wall of the stomach or intestine into the peritoneal cavity. There it had come to rest in the adipose

tissue, in which it was found. Otherwise, an adult may have dropped a first-stage larva on the skin of the host instead of the nostril, and the larva had penetrated through the wall to the body cavity. What would have been its ultimate fate if the host had lived can only be surmised.

According to Brauer (*loc. cit.*), the second and third instars resemble each other in most respects except size and colour of the body and investing spines, and size and colour of the stigmatic plates. In this opinion Brauer was probably in error, and what he considered the second-stage larva was more likely the immature third instar. In order that his descriptions of the larvae should conform with those of earlier authors, Brauer (p. 190), whilst recognising the presence of twelve segments, combined the first body segment with the cephalic as segment I, thus making in all but eleven segments. In clearly recognising twelve segments I prefer to follow Bau (1920, p. 542), according to whom the first or cephalic segment of the larva of *C. ulrichi* is the anterior part of the "cephalic segment" of earlier authors and the true second segment the posterior part. The second segment of previous authors is now the third and so on, the result being that the total number of segments is increased from eleven to twelve by subdividing the former "cephalic segment" into its two component segments. The following is a description of the larva taken at Blair Atholl:

Larva in its early third stadium (Fig. 2 A and B), club-like, 14 mm. long; greatest breadth 5 mm. at the sixth and seventh segments, gradually decreasing from the seventh to the last segment. Dorsal side arched, ventral side flat, lateral surfaces tuberculated. Integument finely granulose. Segment I (cephalic) small, broader than long. Buccal hooks (md.s.) black, abruptly recurved; extremities pointed and laterally divergent, non-denticulate on their concave lower margins. Antennae (ant.) short conical with bases approximate and extremities divergent; apices capped by two minute, dark brown papillae. Segment II twice as broad as long, separated from the first by a furrow clothed with a ruff-like band of small, erect, bristling spines projecting anteriorly like a crown; dorsal surface occupied by a sub-quadrangular naked area (c.), the vertex ("Scheitelfeld") or cervix ("Nackenfeld"); cervical area somewhat broader than long, bounded anteriorly by the opercular furrow, which extends behind to segment V. Three boss-like tubercles laterally on each side of segment II and likewise on the subsequent segments. Anterior spiracles (a.sp.) small, brownish, lateral and dorsal, near the posterior margin of segment II, with free extremities subdivided into short cylindrical lobes, four on the left and six on the right; each lobe terminated by a minute aperture with well-defined brown margin. Segments IV–VII subequal in length. Following segments gradually increase in length up to segment XI, which is the longest. Segment XII smaller, swollen and arched dorsally, terminating below in a posteriorly directed, fleshy proleg, bearing two pairs of small papillae on its upper bare dorsal surface; a transverse row of small spines behind and below; at the extremity, a group of larger spines with the tips bent downwards and forwards.

Posterior stigmata (Fig. 3 D, p.sp.) coarsely porose, dark brown, semi-lunar, obliquely set, with the narrower ventral horns twice as widely separate as the upper broader ones; outer margins with three minute pale spots and slightly scalloped; surface of stigma divided into wedge-shaped areas by unequally spaced, ill-defined lines. False stigma white, of two concentric circles, in the widely open mesial excavation.

All the segments except I, II, XI and XII armed dorsally and ventrally with anterior bands of posteriorly directed spines in irregular rows; each spine set in a broad base and

tapering to a brown point; spines of the posterior smaller than those of the anterior rows. Number of rows on the dorsal surface—three on segment III, four on segments IV–IX, two or three on segment X and a few isolated spines anteriorly on segment XI. Two rows of spines with anteriorly recurved tips project from the posterior margin of segment XI.

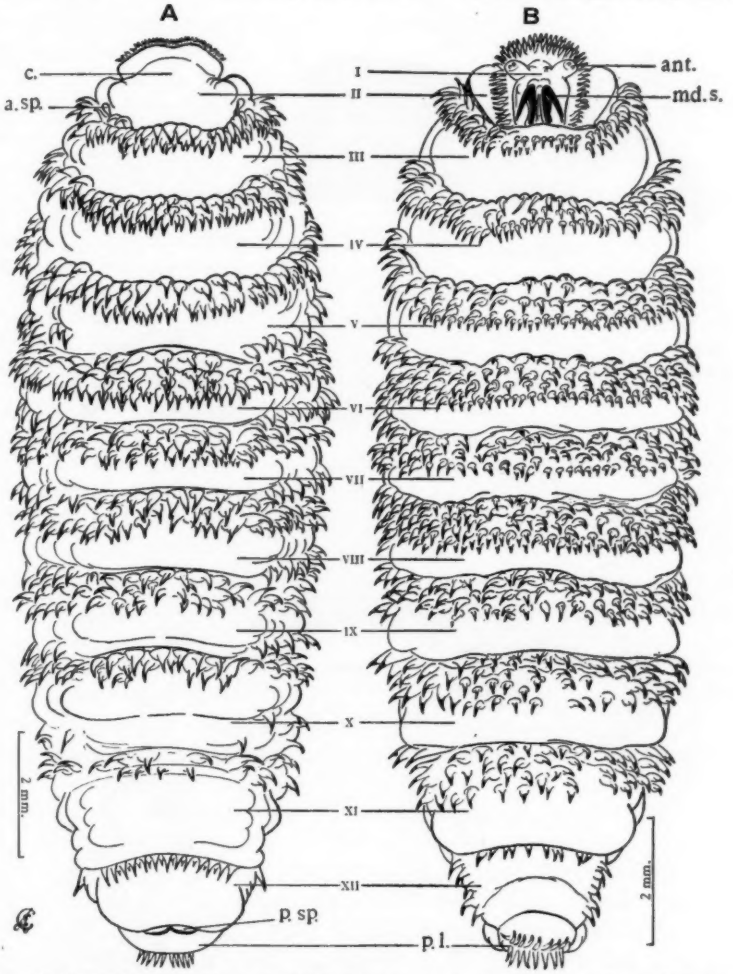

Fig. 2. *Cephenomyia auribarbis*, early third-stage larva. A, dorsal aspect, B, ventral aspect. ant. antenna; a.sp. anterior spiracle; c. cervix ("Nackenfeld"); md.s. mandibilar (buccal) sclerite; p.l. proleg; p.sp. posterior spiracle; I–XII denote segments.

Spinous armature heavier ventrally than dorsally; one or two rows on segment III, three on segment IV, four on segment V, five to seven on segments VI–XI inclusive. On segment XII anteriorly a single row, and a single row on either side in addition to the three transverse rows of anteriorly recurved spines at extremity of proleg. Laterally, on segments V–X, a group of spines behind the others, extending dorsally and ventrally.

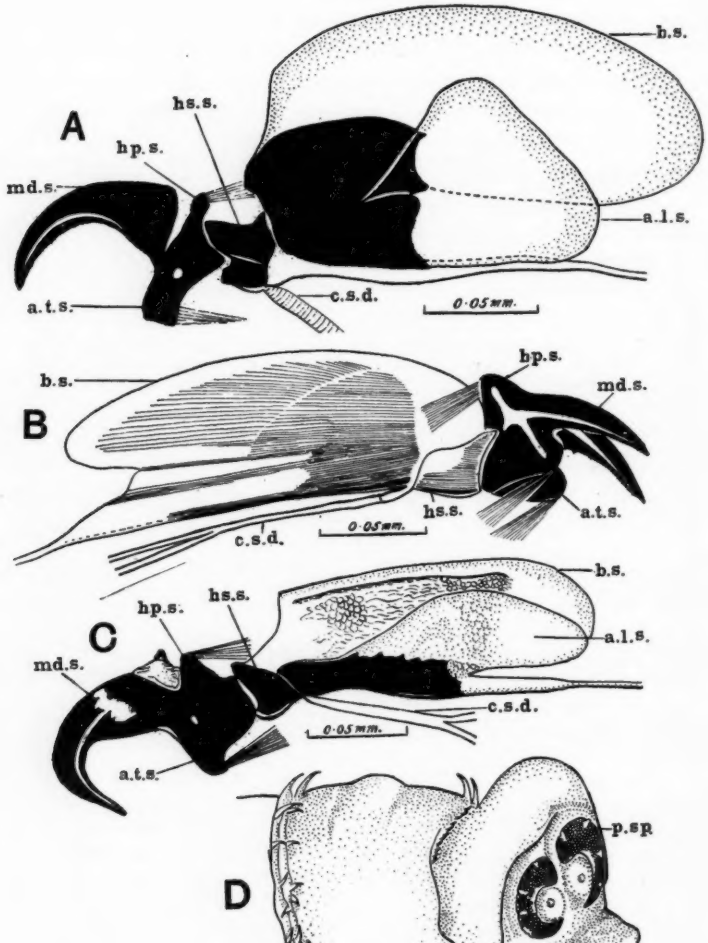

Fig. 3. A. *Cephalomyia* (*Oestrus*) *ovis*
B. *Gastrophilus intestinalis* } Cephalo-pharyngeal apparatus.
C. *Cephenomyia auribarbis*

D. *Cephenomyia auribarbis*, posterior end of larva. a.l.s. accessory lateral sclerite; a.t.s. anterior transverse sclerite; b.s. basilar sclerite; c.s.d. common salivary duct; hp.s. hypopharyngeal sclerite; hs.s. hypostomal sclerite; md.s. mandibular (buccal) sclerite; p.l. proleg ("Nachschieber"); p.sp. posterior spiracle.

It should be noted that Brauer (1863, p. 191) and Bau (1920, p. 547) refer to the proleg ("Nachschieber") as a pushing or propelling organ, which assists the larva ejected from the host in creeping on the ground. Bergman (1917, p. 9), on the other hand, believes its function is one of retarding and attaching the larva to the tissues, when it moves backwards. It is conceivable that it serves both purposes.

The larvae of the four known European species of Cephenomyia can be readily identified by the following key adapted from Bergman by Bau (1920, p. 551):

Larvae in the third stage with a pair of buccal hooks. Posterior stigmata exposed. Antennae approximate at the base ... ... Genus **Cephenomyia** Latreille.

1. Cervical area ("Scheitelfeld" of Brauer) almost quadrangular, slightly longer than broad **2**
   Cervical area rectangular, much longer than broad ... ... **C. stimulator** Clk.
2. Mesial margins of posterior stigmatic plates parallel **3**
   Ventral horns of posterior stigmatic plates divergent ... ... **C. auribarbis** Mg.
3. Posterior stigmatic plates with margin uniform ... ... **C. ulrichi** Br.
   Posterior stigmatic plates with margin undulating ... ... **C. nasalis** L.

V. THE CEPHALO-PHARYNGEAL APPARATUS OF *C. AURIBARBIS*.

The buccal hooks (Fig. 3 C, md.s.) are massive, black, untoothed structures, ventrally recurved and slightly divergent at their pointed, distal extremities. Each hook is a composite structure, the expanded base of which is formed from the fused dorsal hypo-pharyngeal and ventral anterior transverse sclerites (hp.s. and a.t.s.). These sclerites serve respectively for the insertion of the elevator and depressor muscles of the buccal hooks. The hypostomal sclerite (hs.s.) is **V**-shaped in its ventral aspect with a transverse bar uniting the arms just anterior to the aperture for the transmission of the common salivary duct into the buccal cavity. Posteriorly the arms meet behind the aperture and fuse with each other and with the basilar sclerite (b.s.). Anteriorly the divergent arms expand to form broad, antero-ventral surfaces for articulation with the buccal hooks. The basilar sclerite is navicular, heavily chitinised and pigmented along its floor. Laterally, the pigment is more diffuse, and a deep incision partly separates the wall into dorsal and ventral processes. Projecting upwards and outwards from the base there is an accessory or alary process (a.l.s.), which is also present in the basilar sclerite of other species of Cephenomyia, *e.g. C. nasalis*. It is interesting to note that it is also represented in *Cephalomyia* (*Oestrus*) *ovis* (Fig. 3 A), which would suggest that it is correlated in some fashion with the naso-pharyngeal habitat of these two parasitic tachino-oestrid species. Presumably its purpose is to provide a greater area of attachment for the depressor muscles of the buccal hooks, on the contraction of which depends the secure attachment of the larva to the mucous membranes of passages, where violent sneezing by the host might readily cause premature dislodgement with fatal results to the parasite. In the larvae of Gastrophilus (Fig. 3 B), which have only to contend with the more gentle

peristaltic movements of the gut, the risk of accidental dislodgement is small, and the accessory appendage of the basilar sclerite is not developed.

Further comparison of the cephalo-pharyngeal apparatus of *Cephalomyia ovis*, *Gastrophilus intestinalis* and *Cephenomyia auribarbis* shows that in the first the mandibular hook proper is attached to its base (fused hypopharyngeal and anterior transverse sclerites) by a narrow neck only; this is in direct contrast to the broad fusion that occurs in the hooks of the other two species. Of the three, it is noted that *C. auribarbis* alone has hooks that are untoothed. The absence of teeth is, however, offset in some degree by the greater downward curvature of the tip of the hook, which must aid materially in securely anchoring the larva to the mucous membrane of the host.

VI. Summary.

1. *Cephenomyia auribarbis* Mg., a tachino-oestrid parasite of the red deer (*Cervus elaphus* L.), is distributed throughout the deer forests of Scotland. The adult is found on the wing during June and July.

2. The larva normally occurs attached to the walls of the nasal passages and pharynx, where it remains for 10 or 11 months. At maturity it drops to the ground and pupates beneath the surface. The adult emerges in 3–4 weeks.

3. In January, 1931, a larva of the early third instar was recovered from a stag shot at Blair Atholl, Perthshire. It was found in fat in the pelvic region and not in the nostril or pharynx as might have been expected.

4. It is suggested that the larva may have been swallowed by the host and bored its way through the wall of the gut into the peritoneal cavity, whence it had wandered to the pelvic region; or else that a first-stage larva had been accidentally deposited by a female on the hide and had bored through the body wall to the peritoneal cavity.

5. The adult female and the early third-stage larva are described and illustrated.

6. A comparison is made between the cephalo-pharyngeal apparatus of *Cephenomyia auribarbis*, *Cephalomyia ovis*, and *Gastrophilus intestinalis*. It is noted that the basilar sclerite of the first two species is provided with an accessory lateral appendage on each side. A greater surface is thus furnished for the attachment of the large depressor muscles of the buccal hooks. The larva thus securely holds its ground in the nasal cavities and pharynx, where the risks of summary ejection are always present. In *G. intestinalis* the accessory appendage is absent. The chances of the larva of this species losing its hold on the gastric mucous membrane of the host are very slight.

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THE EFFECT OF TEMPERATURE AND HUMIDITY UPON THE SURVIVAL OF CERTAIN UNFED RAT FLEAS.

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(With 4 Charts.)

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I. Previous work.

Rat fleas may be conveyed, apart from the host, from one place to another in merchandise and grain; newly emerged adults must seek a host in order to obtain the blood upon which they feed. Recognition of these facts has caused enquiry to be made from time to time into the duration of life of starved fleas. They have been kept in atmospheres of different temperatures and humidities and the evidence produced is of the utmost importance because of the bearing it has upon the relationship between climatic conditions and the distribution of plague. As the chief vector of plague is the tropical rat flea *Xenopsylla cheopis*, it is upon this species that most work has been done; but research has also been extended to *X. astia*, a rat flea of oriental regions and to *Ceratophyllus fasciatus*, a rat flea of temperate countries.

In 1908, the Advisory Committee on Plague Investigations in India published a report relating to observations on the bionomics of fleas giving special attention to *X. cheopis*. A further report appeared in 1912 on flea breeding in Poona. Then in 1914 Bacot published the results of his experiments in England. After the appearance of Brooks' (1917) paper on plague and climate, wherein he showed that "plague does not maintain itself in epidemic form when the temperature rises above 80° F. accompanied by a saturation deficiency of over 0·30 of an inch," attention began to be directed to saturation deficiency as a factor which might perhaps be directly related to the fleas' length of life. In 1924, Petrie and Todd *et al.*, working in Egypt, published their

investigations relating their results to saturation deficiency. Bacot and Martin's paper (1924), because of its importance, will be referred to again. The work of Hirst (1926) in Colombo and Webster (1930) in Bombay deserve mention. The latest discussion of this subject appeared in a summary of the rat-flea survey of the Madras Presidency by King and Pandit (1931).

The conclusion arrived at by all the investigators is that, under conditions of low temperature and moist atmospheres, fleas survive longer than in surroundings which are hot and dry. Despite this general agreement, attention was not always devoted to (*a*) the measurement of temperature and humidity of the atmosphere in which the fleas were kept; (*b*) the age of the fleas; (*c*) whether the sexes react differently; (*d*) evidence that the fleas were unfed; (*e*) other precise experimental conditions such as identical treatment of individual fleas.

Bacot and Martin's (1924) work undoubtedly approaches nearest to the ideal inasmuch as the atmospheric conditions in which the fleas were kept were accurately controlled, but the insects "represented a mixed population of various ages and in different states of nutrition" and no distinction was made between the sexes. However, they went further than the other workers and found that the survival of *X. cheopis* apart from its host was "approximately in inverse proportion to the saturation deficiency of the air, provided the temperature and air movement are constant"; also, "under similar conditions but with constant saturation deficiency, the length of life is reduced to between one half and two thirds by 10° C. rise in temperature."

II. Methods and source of material.

In the present work efforts have been made to carry out experiments in which all variables but one were controlled. The method employed for maintaining a continuous supply of material and the apparatus devised for collecting the fleas have already been described (Leeson, 1932). The method for obtaining unfed fleas of a known age is explained in the same paper; but it is necessary here to describe in detail the manipulation of the experimental fleas and the arrangements made for controlling temperatures and humidities. The fleas were unfed and not more than 24 hours old. They were collected separately by means of the suction apparatus; then the collecting tube was detached and inverted over another shorter and slightly wider tube. The smaller tube had a diameter of 1½ in. into which the collecting tube was partly inserted when the flea was being transferred; this may appear to be a minor point, but it is really important when handling many fleas. It not only enables the manœuvre to be completed swiftly, but reduces the risk of the flea escaping. To minimise this risk still further the smaller tube was stood in a deep, smooth-sided, white enamel bowl large enough to admit both hands. As each flea was deposited the tube was capped with voile fastened with adhesive tape, numbered, placed in its particular desiccator and registered. The insects were kept singly in the tubes throughout the experiments, so that the treatment accorded

each flea should be as uniform as possible. The tubes were inspected daily; those containing dead fleas were removed; each dead flea was examined to confirm the identification and determine the sex, and all particulars were entered against its serial number in the register.

Temperatures were recorded by thermographs and checked by mercury thermometers. Atmospheres of different percentages of relative humidity were secured inside sealed desiccators by keeping in them the appropriate mixtures of sulphuric acid and water. Pieces of pumice stone were floated in the fluid to prevent splashing and no desiccator was opened oftener than twice in one day, sometimes only once.

It was considered that the introduction of other factors might affect the results in some unknown manner; the fleas were therefore kept in clean glass tubes without material for burrowing or for foothold. Preliminary work showed that when the insects were kept in tubes containing sand or bran they became agitated and liable to injury due to the movement of the material within the tube and it was quite often noticed that the fleas walked on the insides of the gauze caps with which the tubes were covered.

The two species of *Xenopsylla* (*X. cheopis* and *X. astia*) were bred from material brought from Colombo to the London School of Hygiene and Tropical Medicine by Dr L. F. Hirst and arrived on February 24th, 1930. The fleas had travelled in a mixture of bran and dried blood; and from them cultures have been kept going by the methods described elsewhere (Leeson, 1932). On April 24th, 1930, Mr D. A. J. Buxton collected from the London docks a rat's nest which he sent to the School. From this nest originated the culture of *Ceratophyllus fasciatus* which is still flourishing.

Throughout the experiments identical technique has been used and the three species studied side by side.

III. Results of observations.

Xenopsylla cheopis Roths.

All the data regarding the experiments upon this species have been assembled in Table I. The mean survival times have been calculated upon an actuarial basis, as the real time of death was unknown. Fleas seen to be living, say, at the end of the third day's exposure but dead at the next inspection, 24 hours later, are reckoned as having lived 3·5 days and so on. After eliminating all variables as far as possible great inconsistency was still apparent, therefore the standard deviations for all means are included in the table. The fact that they are less at high temperatures does not mean that duration of life is more consistent but depends upon the total periods being shorter.

Before discussing the effects of different atmospheric conditions upon survival it is convenient to analyse the survivals at a single temperature and humidity. Table II shows the frequency distribution of survivals of each sex at 23° C. and 60 per cent. relative humidity. Although the mean survival was

Table I. *Summary of experiments upon* Xenopsylla cheopis *unfed and under 24 hours old at commencement, showing maximum and mean duration of life in days when kept in atmospheres of various temperatures and humidities.*

			Both sexes				Males				Females			
Temp.	% Rel. hum.	mm. Hg sat. deficiency	Number of fleas	Max. survivals in days and sex	Mean survivals in days.	*Standard deviations*	Number of males	Max. survivals in days.	Mean survivals in days.	*Standard deviations*	Number of females	Max. survivals in days	Mean survivals in days.	*Standard deviations*
37° C.	0	46·9	12	2·5♂	1·3	*0·6*	7	2·5	1·5	*0·5*	5	1·5	1·1	*0·5*
or	40	28·1	26	3·5♂	1·7	*0·8*	11	3·5	2·0	*0·9*	15	2·5	1·4	*0·8*
98·6° F.	60	18·8	20	2·5♀	1·3	*0·5*	9	1·5	1·3	*0·4*	11	2·5	1·3	*0·6*
	70	14·1	30	3·5*	1·7	*0·7*	13	3·5	1·5	*0·7*	17	3·5	1·9	*0·7*
	75	11·7	20	2·5♀	1·3	*0·6*	9	1·5	1·1	*0·5*	11	2·5	1·5	*0·6*
	80	9·4	20	3·5♀	1·5	*0·7*	10	2·5	1·6	*0·5*	10	3·5	1·5	*0·8*
	90	4·7	34	3·5♀	1·9	*0·7*	13	2·5	1·7	*0·7*	21	3·5	2·0	*0·7*
30° C.	0	31·7	41	7·5♂	3·5	*1·7*	17	7·5	3·7	*1·7*	24	5·5	3·3	*1·6*
or	35	20·6	60	6·5♂	3·7	*1·4*	43	6·5	3·8	*1·5*	17	4·5	3·5	*1·3*
86° F.	50	15·9	107	8·5*	4·2	*1·5*	59	8·5	4·5	*1·6*	48	8·5	3·8	*1·5*
	60	12·7	146	17·5♀	5·3	*2·7*	92	10·5	4·9	*2·2*	54	17·5	5·9	*3·0*†
	75	7·9	68	10·5♂	4·4	*1·8*	34	10·5	4·7	*2·2*	34	6·5	3·8	*2·2*
	85	4·8	75	13·5♂	4·7	*2·4*	45	13·5	4·9	*2·9*	30	7·5	4·2	*1·4*†
	95	1·6	85	11·5♂	4·4	*2·2*	49	11·5	4·7	*2·5*	36	7·5	4·4	*1·7*
23° C.	0	21·0	60	6·5♂	3·7	*1·7*	27	6·5	3·4	*1·9*	33	5·5	3·9	*1·5*
or	30	14·7	154	13·5♂	4·8	*2·3*	87	13·5	5·1	*2·6*	67	9·5	4·4	*2·1*
73·4° F.	40	12·6	172	13·5♀	4·9	*2·6*	100	10·5	4·7	*2·4*	72	13·5	5·2	*2·8*
	60	8·4	113	14·5♂	6·2	*2·6*	60	14·5	6·2	*2·8*	53	12·5	6·2	*2·3*
	75	5·3	98	13·5*	6·5	*2·7*	50	13·5	6·5	*2·6*	48	13·5	6·5	*2·8*
	90	2·1	140	21·5♂	7·3	*4·4*	70	21·5	6·3	*3·9*	70	20·5	8·3	*4·6*†
	95	1·1	123	15·5♂	6·5	*2·7*	74	15·5	6·4	*2·8*	49	11·5	6·5	*2·1*
18° C.	0	15·5	47	9·5♂	4·8	*2·1*	22	9·5	5·2	*2·2*	25	8·5	4·4	*1·9*
or	20	12·4	54	16·5♂	6·9	*2·6*	27	16·5	7·5	*2·6*	27	11·5	6·5	*2·4*
64·4° F.	75	3·9	127	32·5♂	13·3	*6·9*	83	32·5	14·1	*6·8*	44	29·5	12·0	*6·7*
	90	1·6	96	61·5♂	14·6	*8·6*	49	61·5	16·5	*9·0*	47	23·5	12·5	*7·8*
12° C.	0	10·5	4	19·5♂	14·8	*2·8*	2	19·5	16·5	*3·0*	2	13·5	13·0	*0·5*
or	60	4·2	10	30·5♀	17·1	*5·2*	5	19·5	15·7	*2·8*	5	30·5	18·5	*6·5*
53·6° F.	75	2·7	31	30·5♂	19·1	*6·8*	24	30·5	20·0	*6·6*	7	26·5	15·8	*5·5*
	90	1·1	54	37·5♂	21·3	*7·5*	23	37·5	23·2	*9·0*	31	32·5	20·1	*4·9*†
			2027				1114				913			

* Indicates that individuals of both sexes survived equally.

† Indicates those survivals where one sex lives longer than the other, but although the differences are statistically significant they do not relate to one sex alone.

Table II. *Frequency distribution of survival of unfed* Xenopsylla cheopis *kept at* 23° C. *and* 60 *per cent. relative humidity; males and females shown separately.*

	Days survived															Total fleas
	0–1	–2	–3	–4	–5	–6	–7	–8	–9	–10	–11	–12	–13	–14	–15	
No. of ♂♂	2	1	3	5	9	11	9	7	5	0	4	3	0	0	1	60♂♂
No. of ♀♀	1	2	3	3	5	7	12	9	7	2	1	0	1	0	0	53♀♀
No. of both sexes	3	3	6	8	14	18	21	16	12	2	5	3	1	0	1	113

in each case 6·2 days, there is no striking concentration around the mean. Eleven males were dead at the end of 6 days' exposure, but it was also possible for one individual to exist until the fifteenth day. The female figures are slightly different; one flea lived until the thirteenth day and it will also be observed that two males and one female died during the first day. This distribution is fairly typical of the other experiments. There is, however, one extreme instance of a flea surviving for 61–62 days; this was at 18° C. and 90 per cent. relative humidity; the next longest survival at this temperature and humidity was 29–30 days. In nearly every case one or more fleas died after the first day's exposure and an idea of the spread of the survival times can be obtained from the figures in Table I under the heading "maximum survival and sex."

Sex.

The figures in this column convey the impression that there is no difference between the reaction of the sexes. A few authors have recorded that such a distinction exists; Bacot (1914), for example, writing of fleas which had been fed noted that long-lived specimens were always females. Hirst (1926) found that females lived longer than males, and Webster's (1930) experiments with infected fleas gave the same result, but when he used newly emerged fleas males frequently outlived females.

No author gives any measure of consistency by which the longevity of the sexes could be compared statistically; but from Table I, where the number of observations is sufficient, it can be shown that there is no significant difference between the distributions of the sexes by expressing the standard deviations as percentages of the means. For example, at 23° C., the coefficients of variation thus calculated would be: at 30 per cent. relative humidity, male 51, female 48 and at 75 per cent., male 40, female 43. It will be further noticed that those differences between survival times which are significant do not relate to one sex alone. These instances are marked † in the table.

One may conclude that whatever may be the case with fed fleas, the sex of unfed fleas does not influence their survival. Neither can any relationship be found between the survival of the sexes and any particular temperature or humidity. Sex, then, may be disregarded and males and females considered together in examining the effect of temperature and humidity upon them.

Humidity.

The mean survival times for both sexes given in Table I are plotted in Chart 1; those at the same temperature are connected. The figure for 0 per cent. relative humidity and 12° C. may be too high, as it is derived from the survivals of four fleas only.

There are three points worthy of note in Chart 1. Firstly, it is obvious that the fleas survive longer at lower temperatures. Secondly, that at any given temperature survivals are greater at higher humidities with a possible optimum

of about 90 per cent. The third point qualifies the second, inasmuch as at higher temperatures there is not only a shortening of life due to heat itself but there would appear to be a reduction in the extent to which moisture can influence survival; but it must be remembered that at low temperatures the difference due to humidity is easy to see, because it is a relatively long time. The same proportional difference at high temperatures would be fewer hours and would look less.

At 30° C. the increases in longevity as humidity is raised are very small; at 37° C. they have practically disappeared, so that it seems that at this temperature humidity has no effect upon the life of the insect.

As the length of life is so limited and observations were made daily, the margin of time is too restricted to allow of small differences in longevity to

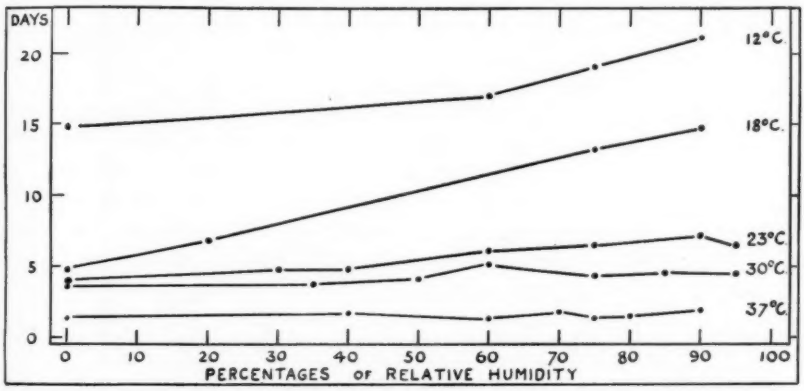

Chart 1. Mean survivals in days of unfed fleas (*Xenopsylla cheopis*) at different humidities and temperatures; survivals at the same temperature are connected by lines.

be recorded. To remedy this it is necessary to make investigations at this temperature by inspections at shorter intervals. This has been done by Mellanby (1932), but only at sub-lethal temperatures. His conclusions are in accord with mine. He showed that unfed adult *X. cheopis* were able to withstand a temperature no higher than 40·5° C. for 1 hour and 37·8° C. for 24 hours at all humidities; the insects were dead at the end of these times when exposed to any higher temperatures regardless of the humidity of the surrounding atmosphere.

It appears that the influence of humidity on the life of the fleas is greatest at or about 18° C., gradually diminishing in power as the temperature rises or falls from this point. Reference to Table I shows that when survivals are compared with the relative scale of humidity, at 37° C. the mean survival is about 1·5 days whatever the humidity up to 90 per cent.; at 30° C. there is an increase from 3·5 to 4·4 days through the same range; at 23° C. the increase is from 3·7 to 7·3 days (about twice as long); at 18° C. the figures are

respectively 4·8 and 14·6 days (about three times as long); then at 12° C. the mean survival at 90 per cent. drops to something over twice as long.

If the survivals at 0 per cent. relative humidity are multiplied by the factor shown in Table III the product is the survival in days at 90 per cent. relative humidity. The factor falls with rising temperature except that it is less at 12° C. than at 18° C. At some temperatures below 18° C. fleas may live longer than they do at 18° C., but the influence of humidity in prolonging life is proportionately weaker. The temperature at which fleas will live for the maximum period may be much lower than 18° C., but the proportional increase in longevity for every degree fall of temperature is steadily reduced.

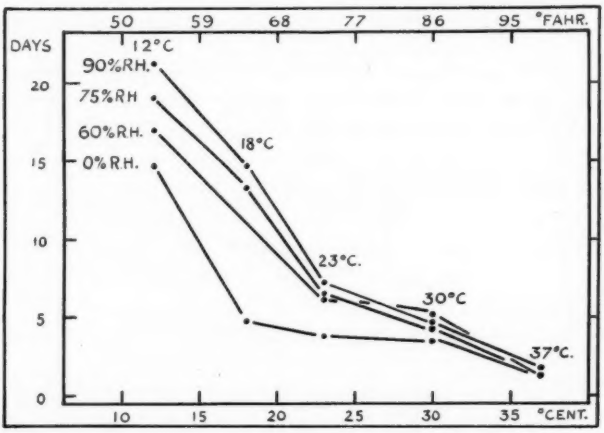

Chart 2. Mean survivals in days of unfed fleas (*Xenopsylla cheopis*) at different temperatures and humidities; survivals at the same humidities are connected by lines.

Table III. *Mean survivals in days of unfed* Xenopsylla cheopis *at* 0 *per cent. relative humidity multiplied by the factor shown gives mean survivals at* 90 *per cent. relative humidity.*

Temp. ° C.	Mean survivals in days at 0 % rel. hum.	Factor (approx.)	Product = mean survivals at 90 % rel. hum. (days)
37	1·5	1·0	1·5
30	3·5	1·25	4·4
23	3·7	2·0	7·3
18	4·8	3·0	14·6
12	9·0*	2·3	21·3

* This figure is found by interpolation as the survivals at 12° C. and 0 per cent. relative humidity were derived from four fleas only.

Chart 2 illustrates the point in another manner. In this chart the survivals at the same relative humidities are connected by lines and it will be seen that at 18° C. the increase in length of life is greatest. The points at temperatures above and below 18° C. are nearer so that the connecting lines form a curve.

Whether humidity would again become ineffective at some lower temperature demands further investigation. However, when the survivals are examined in their relation to saturation deficiency quite a different result is found.

Saturation deficiency.

In the preceding pages the relative notation for measuring humidity has been used partly because it is more familiar to most readers and partly because the mixtures of sulphuric acid and water employed to control humidity give one a particular relative humidity, almost irrespective of temperature.

It will be remembered, however, that Brooks (1917) showed that there was a relationship between epidemics of plague and saturation deficiency, also that Bacot and Martin (1924) found that the survival of fleas was directly proportional to saturation deficiency at one temperature. In other words, it is proportional to the rate at which they lose water, though the authors noted that the proportion broke down at high humidities. This exception is of course to be expected; they also found that there is no direct relation between saturation deficiency and duration of life at different temperatures, this being due to the fact that the temperature itself makes so much difference.

Moreover, Buxton (1931 *a*), applying the law of saturation deficiency to data concerning the survivals of quite unrelated insects and collected by many authors, showed that one of the causes of death is loss of water and that the rate of this loss of water is determined by saturation deficiency.

It is therefore proposed to examine the data accumulated in the present experiments in order to discover whether the survival of unfed fleas is proportional to relative humidity or to saturation deficiency. It may be proportional to neither but cannot be proportional to both, for as atmospheres of high temperatures are able to hold more water vapour than atmospheres of low temperatures, saturation deficiencies of atmospheres of equal relative humidity are different at different temperatures.

A glance at Table I shows that there is no simple measure of duration in terms of humidity if several temperatures are compared. For example:

30° C. and 75 per cent. relative humidity duration = 4·4 days
23° C. „ 75 „ „ „ = 6·5 „
18° C. „ 75 „ „ „ = 13·3 „

or,

30° C. and 12·7 mm. saturation deficiency duration = 5·3 days
23° C. „ 12·6 „ „ „ = 4·9 „
18° C. „ 12·4 „ „ „ = 6·9 „

Also the fact that the lines in Chart 1 are straight and in Chart 2 curved indicates that temperature determines survival with very little regard to humidity by any measure.

But if the survivals at only one temperature are considered one may

examine the relationship between saturation deficiency and duration of life by two methods.

The first is arithmetical. As other insects do not follow the saturation deficiency law in saturated or very dry air (Buxton, 1931 *a*), it is legitimate to take as the basis of calculation the survival time at 60 per cent. relative humidity. The experiments at 23° C. will be taken as an example. The saturation deficiency at 60 per cent. relative humidity is 8·4 mm. and the mean survival 6·2 days. If survival was directly proportional to saturation deficiency, the expected duration at other saturation deficiencies could be found by dividing the product of these two figures by the other saturation deficiencies. In Table IV the figures are set out showing actual mean survivals and the calculated "expected" survivals. It is obvious that no relationship exists, neither does it exist at any of the other temperatures employed.

The second method of examining this question is graphical. Chart 3 is an adaptation of Buxton's Fig. 2 (1931 *b*) for the conversion of relative humidities

Table IV. *Mean and "expected" survivals in days of unfed* Xenopsylla cheopis *at* 23° C. *and different humidities.*

Relative humidity %	Saturation deficiency mm.	Mean survivals (days)	"Expected" duration of life (days)
0	21	3·7	2·5
30	14·7	4·8	3·5
40	12·6	4·9	4·1
60	*8·4*	*6·2*	*6·2*
75	5·3	6·5	9·8
90	2·1	7·3	24·8
95	1·1	6·5	47·3

The "expected" survivals are arrived at by dividing the product of the saturation deficiency figure and the mean survival at 60 per cent. by the respective saturation deficiencies. The result shows that there is no direct relationship between survival and saturation deficiency.

at known temperatures to saturation deficiencies. The rectilinear axes are temperature and relative humidity; the curved lines join points of equal saturation deficiency. On the graph are shown the actual figures obtained in these experiments and the broken lines represent expected survival times which have been interpolated. These lines are very far from being parallel to lines of equal saturation deficiency and are nearly horizontal showing dependence mainly on temperature.

If temperature be taken as the constant the effect of changing the saturation deficiency from 2 to 10 mm. at 12° C. would produce a probable diminution of 10 days in the survival time (20–10 days), but at 21° C. the same alteration in the saturation deficiency causes a reduction of only 4 days, that is from 10 to 6 days. At 37° C. no change in saturation deficiency has any effect upon the survival of the fleas.

Bacot and Martin (1924) showed that duration of life of *X. cheopis* was directly related to saturation deficiency at 32° C. The present experiments

show equally clearly that it is not, at any temperature; not even at 30 and 37° C., the temperatures which most nearly approach theirs. The only difference between the experiments which could perhaps account for so great a difference in result is the fact that their fleas had fed before the experiments commenced. Fleas which have had at least one feed previous to being starved react differently from those which have never fed at all. Bacot (1914), referring to *P. irritans* and *C. fasciatus*, noted that "under warm to high temperatures with a low humidity, a meal to start with is a very valuable asset."

Table V. *Summary of experiments upon* Xenopsylla astia *unfed and under 24 hours old at commencement, showing maximum and mean duration of life in days when kept in atmospheres of various temperatures and humidities.*

			Both sexes				Males				Females			
Temp.	% rel. hum.	mm. Hg sat. deficiency	Number of fleas	Max. survivals in days and sex	Mean survivals in days.	*Standard deviations*	Number of males	Max. survivals in days.	Mean survivals in days.	*Standard deviations*	Number of females	Max. survivals in days.	Mean survivals in days.	*Standard deviations*
30° C.	0	31·7	36	7·5♂	4·8	*1·8*	22	7·5	5·1	*2·2*	14	6·5	4·4	*1·2*
or	30	22·2	45	6·5*	4·0	*1·4*	20	6·5	4·1	*1·5*	25	6·5	3·9	*1·4*
86° F.	50	15·9	34	5·5*	4·4	*1·9*	7	5·5	3·5	*1·7*	27	5·5	4·6	*1·2*
	60	12·7	124	8·5♂	4·1	*1·4*	76	8·5	4·3	*1·5*	48	6·5	3·8	*1·3*
	75	7·9	36	15·5♂	6·0	*2·3*	25	15·5	6·1	*2·5*	11	10·5	5·7	*1·9*
	90	3·2	58	13·5♀	4·7	*1·8*	32	7·5	4·5	*1·6*	26	13·5	5·0	*2·0*
23° C.	0	21·0	27	9·5♂	4·0	*2·2*	13	9·5	4·7	*2·5*	14	5·5	3·4	*1·2*
or	30	14·7	39	11·5♀	5·6	*2·2*	17	10·5	5·5	*2·1*	22	11·5	5·6	*2·4*
73·4° F.	60	8·4	162	17·5♂	7·2	*3·1*	76	17·5	7·6	*3·6*	86	14·5	6·8	*2·8*
	75	5·3	143	19·5♀	8·8	*4·0*	74	18·5	9·2	*3·8*	69	19·5	8·3	*4·3*
	90	2·1	163	21·5♀	7·9	*4·7*	99	20·5	7·0	*4·0*	64	21·5	9·3	*5·2*
	95	1·1	21	15·5♂	6·7	*3·0*	18	15·5	6·7	*2·8*	3	10·5	7·1	*3·0*
18° C.	0	15·5	36	14·5♂	6·1	*3·2*	16	14·5	8·4	*3·3*	20	6·5	4·3	*1·2*
or	30	10·9	10	19·5♂	12·0	*3·7*	7	19·5	13·2	*2·9*	3	14·5	9·1	*4·0*
64·4° F.	75	3·9	24	24·5♀	11·5	*5·6*	14	18·5	12·9	*3·6*	10	24·5	9·6	*6·4*
	90	1·6	41	32·5♂	15·1	*7·3*	19	32·5	20·3	*6·4*	22	17·5	10·6	*4·5*
			999				535				464			

* Indicates that individuals of both sexes survived equally.

Xenopsylla astia, Roths.

By employing the same methods in analysing the data given in Table V relating to this species, it is found that all that has been said regarding *X. cheopis* applies equally well to *X. astia*.

Survival of unfed *X. astia* is not influenced by sex, though Hirst (1926) found that there was a striking difference between the longevity of the two sexes. His fleas were collected off rats and, as Bacot (1914) showed for other species, fed fleas react differently from fleas which have never fed. That this is so is corroborated by Webster's (1930) experiments in Bombay when he found that infected *X. astia* females usually outlived the males, but that with unfed fleas the males in several instances had longer periods of survival.

Hirst also records that *X. cheopis* has a longer mean duration of life than *X. astia* under Colombo conditions, though he states that his figures are some-

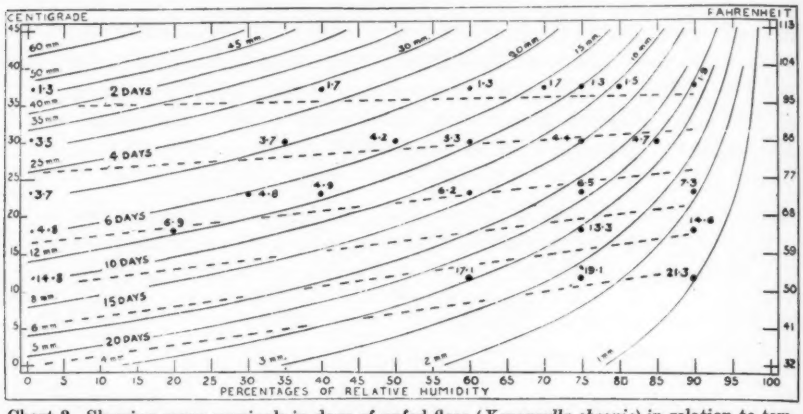

Chart 3. Showing mean survivals in days of unfed fleas (*Xenopsylla cheopis*) in relation to temperature, relative humidity and saturation deficiency. Broken lines indicate interpolated survival times.

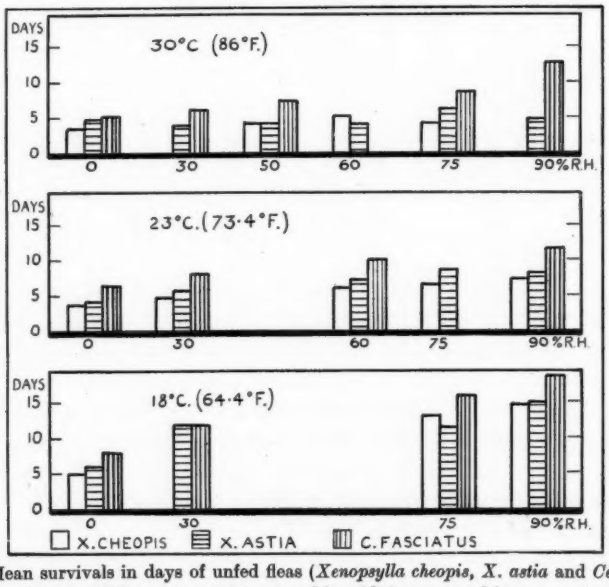

Chart 4 Mean survivals in days of unfed fleas (*Xenopsylla cheopis*, *X. astia* and *Ceratophyllus asciatus*) at different temperatures and humidities, grouped for comparison.

what small for the purposes of such a comparison. On comparing the survivals of *X. astia* and *X. cheopis* in the present experiments, there is some evidence that the former species is able to survive the longer (Chart 4). The difference

between my results and those of Hirst may be due to differences in the experimental methods. However, Webster (1930), experimenting with *X. brasiliensis* in addition to the other two species, found no difference in the longevity of the three species.

Table VI. *Mean survivals of unfed* Xenopsylla astia *at different temperatures and relative humidities of* 0 *per cent. and* 90 *per cent., showing ratio between them.*

Temp. °C.	Mean survivals in days at 0 % rel. hum.	Factor (approx.)	Mean survivals in days at 90 % rel. hum.
30	4·8	1·0	4·7
23	4·0	1·9	7·9
18	6·1	2·5	15·1

Note. The ratio increases in value as the temperature falls, showing the greater influence of humidity at low temperatures.

Table VII. *Summary of experiments upon* Ceratophyllus fasciatus *unfed and under* 24 *hours old at commencement, showing maximum and mean duration of life in days when kept in atmospheres of various temperatures and humidities.*

			Both sexes				Males				Females			
Temp.	% rel. hum.	mm. Hg sat. deficiency	Number of fleas	Max. survivals in days, and sex	Mean survivals in days	*Standard deviations*	Number of males	Max. survivals in days	Mean survivals in days	*Standard deviations*	Number of females	Max. survivals in days	Mean survivals in days	*Standard deviations*
30° C.	0	31·7	70	9·5*	5·2	*2·0*	29	9·5	5·1	*2·0*	41	9·5	5·3	*2·0*
or	30	22·2	34	10·5♀	6·3	*2·0*	17	9·5	5·5	*1·9*	17	10·5	7·0	*2·2*
86° F.	50	15·9	54	11·5*	7·5	*2·6*	22	11·5	8·1	*2·1*	32	11·5	7·1	*2·2*
	75	7·9	5	10·5♀	8·7	*2·4*	—	—	—	—	5	10·5	8·7	*2·4*
	90	3·2	49	26·5♂	12·8	*5·3*	23	26·5	12·6	*4·2*	26	24·5	13·0	*5·4*
23° C.	0	21·0	66	12·5♂	6·5	*2·5*	37	12·5	7·1	*2·7*	29	11·5	5·8	*2·1*
or	30	14·7	57	24·5♂	8·1	*3·6*	21	24·5	8·3	*4·7*	36	14·5	8·0	*3·1*
73·4° F.	60	8·4	66	30·5♀	10·1	*5·0*	37	19·5	10·4	*4·4*	29	30·5	9·8	*5·6*
	90	2·1	102	26·5♂	11·7	*6·7*	47	26·5	11·1	*6·7*	55	23·5	12·1	*6·7*
18° C.	0	15·5	31	15·5*	8·0	*3·7*	13	15·5	9·3	*3·6*	18	15·5	7·1	*3·4*
or	30	10·9	13	19·5♀	11·9	*2·9*	5	12·5	11·3	*1·2*	8	19·5	12·3	*3·5*
64·4° F.	75	3·9	46	32·5♂	16·3	*7·3*	21	32·5	17·6	*8·2*	25	26·5	15·3	*6·2*
	90	1·6	53	39·5♂	18·8	*9·3*	25	39·5	22·2	*10·9*	28	27·5	15·7	*6·0*
			646				297				349			

* Indicates that individuals of both sexes survived equally.

In Table VI is shown the ratio which exists between the survivals at 0 and 90 per cent. relative humidity at the three temperatures. The proportion increases as the temperature is lowered, thus showing the steadily increasing influence of humidity at lower temperatures. Here again *X. astia* closely resembles *X. cheopis* (Table III).

Expected survivals calculated as in the case of *X. cheopis* (Table IV) show that with *X. astia* there is also no direct proportion between saturation

deficiency and survival. This view is supported by plotting the survival times in the same way as in Chart 3.

Ceratophyllus fasciatus Bosc.

The data regarding this species are summarised in Table VII. If they are analysed in the same way as has been done for the two species of *Xenopsylla*, they lead one to the same conclusions.

Apart from the general agreement, the main fact emerging from the experiments upon this species is that under similar conditions *Ceratophyllus fasciatus* was able to exist for longer periods than the other two species (see Chart 4).

IV. Summary.

Previous work on the effect of temperature and humidity upon the survival of unfed fleas is criticised in respect of experimental technique. In the present investigation measures have been taken to eliminate as many variables as possible and the methods employed to this end are described.

In the experiments, 2027 *Xenopsylla cheopis*, 999 *X. astia* and 646 *Ceratophyllus fasciatus* were employed, all unfed and less than 24 hours old at the commencement of the different experiments. Records were kept individually so that a statistical measure of consistency could be made.

The following conclusions have been reached:

(1) Duration of life of unfed fleas is not influenced by sex.

(2) High temperatures and low humidities tend to shorten life; conversely, low temperatures and high humidities, with a possible optimum of about 90 per cent., tend to produce longer lives.

(3) Humidity is at its maximum efficiency in this respect at about 18° C.

(4) At 37° C. humidity ceases to have any effect upon the duration of life of unfed fleas, and the evidence suggests that there is also a low temperature at which humidity has no influence upon survival.

(5) There is no direct proportion between survival of unfed fleas and saturation deficiency of the atmosphere at any temperature.

Comparing the species, it was found that:

(1) *Ceratophyllus fasciatus* was the longest lived, at similar atmospheric conditions.

(2) *Xenopsylla astia* lived longer than *X. cheopis* at all humidities at 23° C.

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ON PROTOZOAL BLOOD PARASITES COLLECTED IN UGANDA.

WITH AN ACCOUNT OF THE LIFE CYCLE OF THE CROCODILE HAEMOGREGARINE.

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(With 56 Figures in the Text.)

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INTRODUCTION.

In the present paper an account is given of some protozoa from the blood of various vertebrates collected in Uganda, between 1927 and 1929, while I was attached to the Human Trypanosomiasis Institute, Entebbe. Though most of the time was occupied in the investigation of other problems, every opportunity was taken to examine the representatives of the local fauna for parasitic protozoa. The majority of the animals were captured by myself or by natives and brought to the laboratory alive; others were obtained while on safari[1].

[1] Valuable assistance was rendered to me on these and other occasions by various government officers. I wish in particular to acknowledge my indebtedness to Captain C. R. S. Pitman, D.S.O., M.C., Game Warden of the Protectorate, whom I accompanied on safari to the Bugerere district. Captain Pitman's extensive knowledge of the African fauna facilitated my task considerably. I am also grateful to Mr G. C. Whitehouse, Officer i/c Sese Islands, with whom I visited the islands, and to Mr R. A. Gibson, Acting Conservator of Forests, in whose company I visited the Katera Forest. On my return to England the host animals were kindly identified by Miss J. St Leger, and Messrs J. R. Norman and H. W. Parker, of the British Museum (Natural History Department), to whom my thanks are due.

The laboratory facilities available in Entebbe enabled me to make the best use of the material at my disposal. Only freshly killed or living animals were used and the preparations were examined on the spot. It was thus possible to reject all the negative material, with the result that the whole collection brought home for further study consisted of preparations known to contain parasites.

The total number of vertebrate animals examined was 365, representing about 60 distinct species. Of this number 129 were mammals, 41 birds, 148 reptiles, 22 amphibia and 16 fishes. Protozoan parasites (from the blood and intestine) were recovered from 124 individuals. In this paper it is proposed to deal only with the blood parasites. These were obtained from 31 species of animals distributed as follows: fish 5; amphibia 1; reptiles: lizards 3, crocodiles 1, snakes 9, tortoises 1; birds 4; mammals 7. Two of the new parasites were described elsewhere (Hoare, 1930, 1930*a*).

A brief description is given of all the new forms, and of known parasites reported for new hosts. In the case of the haemogregarine of the crocodile, observations have been made which throw new light on its life cycle and systematic position. Parasites previously described and found by me in the same host are merely recorded in the general list.

In all cases the material consisted of blood films and organ smears stained by Giemsa's method, and of organs fixed in Dobell's modification of Bouin's picro-formol which is the most suitable fixative for field work.

DESCRIPTION.

I. PISCES.

A trypanosome of Haplochromis *spp.*

About fifteen specimens of small fish from Victoria Nyanza, belonging to the genus *Haplochromis* (Cichlidae), and known locally by the Luganda name "enkeje," were examined. The blood of some of these fish harboured a *Dactylosoma* (*D. mariae* Hoare 1930) and a trypanosome. The sporozoon has already been described elsewhere (Hoare, 1930).

The trypanosome occurred in very small numbers in the following species: *H. nubilus*, *H. serranus*, *H. cinereus* and *H. humilior*. In the first three hosts it was found together with the *Dactylosoma*, while the last named harboured the trypanosome alone.

This parasite (Fig. 1) is a typical fish trypanosome in appearance. It has an elongated vermiform body, measuring from 40 to 55μ in length, with the nucleus near the middle of the body and the kinetoplast at the pointed posterior end. The undulating membrane is strongly developed and a short free flagellum is present.

As trypanosomes have not hitherto been recorded from members of this genus of fish, I propose to name it *Trypanosoma mukasai* n.sp.[1]

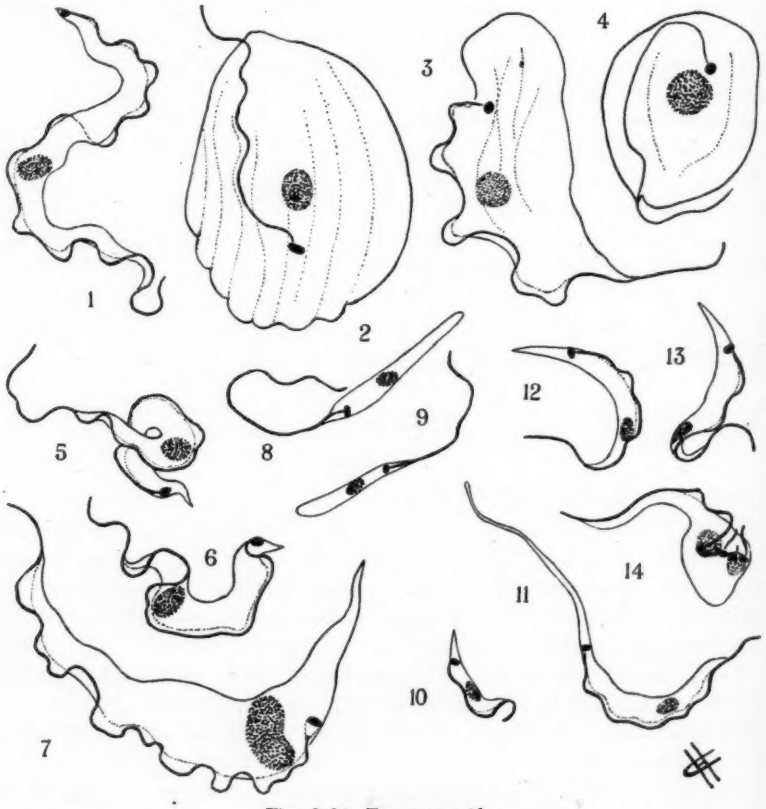
[1] "Mukasa" is the Lake god in the mythology of the Baganda tribe.

II. Amphibia.

A number of toads (*Bufo regularis*) and tree frogs (Hylidae) were examined for blood parasites with negative results.

A trypanosome and a haemogregarine of Hyperolius *sp.*

In the blood of a small frog, *Hyperolius* (sp. indet.) (Cystignathidae), caught on Bugala, an island of the Sese group, a fair number of trypanosomes and a few haemogregarines were found.



Figs. 1–14. Trypanosomidae.
1, *Trypanosoma mukasai* n.sp. from fish. 2, 3, *Trypanosoma* sp. from frog. 4, *T. martini* from skink. 5, 6, 7, *T. perodictici* from potto. 8, 9, *Leishmania* sp. from chamaeleon. 10–14, *T. lewisi* from rat. (×1500.)

The trypanosome (Figs. 2, 3) is very similar to *Trypanosoma rotatorium*. The forms encountered are broad, leaf-like, measuring 30 × 23μ, or somewhat more slender, measuring 43 × 13μ. As *T. rotatorium* has a cosmopolitan

distribution amongst various frogs, it is probable that the present parasite belongs to this species.

The haemogregarines are represented by vermicular forms doubled up in the red cells (Fig. 24) or free in the blood (Fig. 23), measuring about 31·5 × 1·3μ, and by broad sausage-shaped forms (Fig. 25) which appear to result from fusion of the two limbs of the looped forms. These forms measure 17·3×6·6μ. It is proposed to name this parasite *Haemogregarina hyperolii* n.sp.

III. REPTILIA.

(*a*) *Lacertilia.*

The following species of lizards were examined for blood parasites with negative results: *Agama atricollis, Chamaeleo gracilis, C. ellioti, Chamaesaura* sp., *Feylinia currori, Hemidactylus brooki.*

A Leishmania *from a chamaeleon.*

Though no parasites were recovered from the blood of *Chamaeleo ellioti*, the rectum and cloaca of a number of individuals were heavily parasitised by a Leishmania (Figs. 8, 9). These flagellates have not hitherto been recorded from this chamaeleon. They do not, however, differ in any respect from *L. chamaeleonis* described from *C. pumilus* and *C. vulgaris.*

A Plasmodium *and a trypanosome from skinks.*

The only blood parasites of any interest seen in the lizards were obtained from two species of skink, *Mabuya striata* and *M. maculilabris*, caught on the Sese Islands and in the Bugerere district. The six specimens of *M. striata* examined harboured a Plasmodium, while in one a trypanosome was also found. Only one out of the three *M. maculilabris* examined was infected with a Plasmodium, identical with the preceding one.

All the stages of asexual development of the parasite were seen in the blood. The smallest forms are spindle-shaped or irregular, measuring about 3 × 1·5μ (Figs. 15, 16), and are devoid of pigment. They are evidently the trophozoites. Larger forms (Figs. 17–19), measuring from 5·5 × 2·7 to 10 × 4μ, represent dividing schizonts. I have not seen the final stages of schizogony, but from the number of nuclei in the mature schizonts (Fig. 19) it would appear that about ten merozoites are produced. The schizonts contain a distinct mass of pigment granules. There are two other stages of this parasite, evidently representing the gametocytes. One is bean-shaped, measuring 16 × 4·6μ (Fig. 21), the other vermicular, 16 × 2·3μ (Fig. 22), both containing pigment granules. What appears to be a young gametocyte is shown in Fig. 20. It measures 6·6 × 4·6μ, and, except that its nucleus is undivided, it is similar in appearance to a schizont.

Similar parasites were described by Schwetz (1931) from *Mabuya maculilabris*, from the Belgian Congo, under the name *Plasmodium maculilabre*, and

by Wenyon (1909) from *M. quinquetaeniata*, from the Sudan, under the name *P. mabuiae*.

The parasite described in the present paper differs from *P. maculilabre* in the smaller size of the fully developed schizonts, which occupy only a small part of the host cell, whereas those of *P. maculilabre* fill almost the entire cell. Moreover, in the former only ten merozoites are produced, as compared with about twenty in the latter parasite. On the other hand, all the stages of *P. mabuiae*, especially the gametocytes, are considerably smaller in size than the corresponding stages of the parasite described here. The number of merozoites (six) produced by *P. mabuiae* is also smaller.

There seem to be sufficient grounds for recognising this parasite as a distinct species for which the name *Plasmodium pitmani* n.sp. is proposed, in honour of Captain C. R. S. Pitman, D.S.O., M.C.

The trypanosome found in one individual of *M. striata* is similar to the leaf-like stages of *Trypanosoma rotatorium*, and is probably identical with *T. martini* Bouet 1909, from the Ivory Coast, which was also recorded by Schwetz (1931) from the Belgian Congo, in both cases in *M. maculilabris*. The trypanosome of *M. striata* (Fig. 4) measures about $20 \times 16\mu$.

(*b*) *Ophidia.*

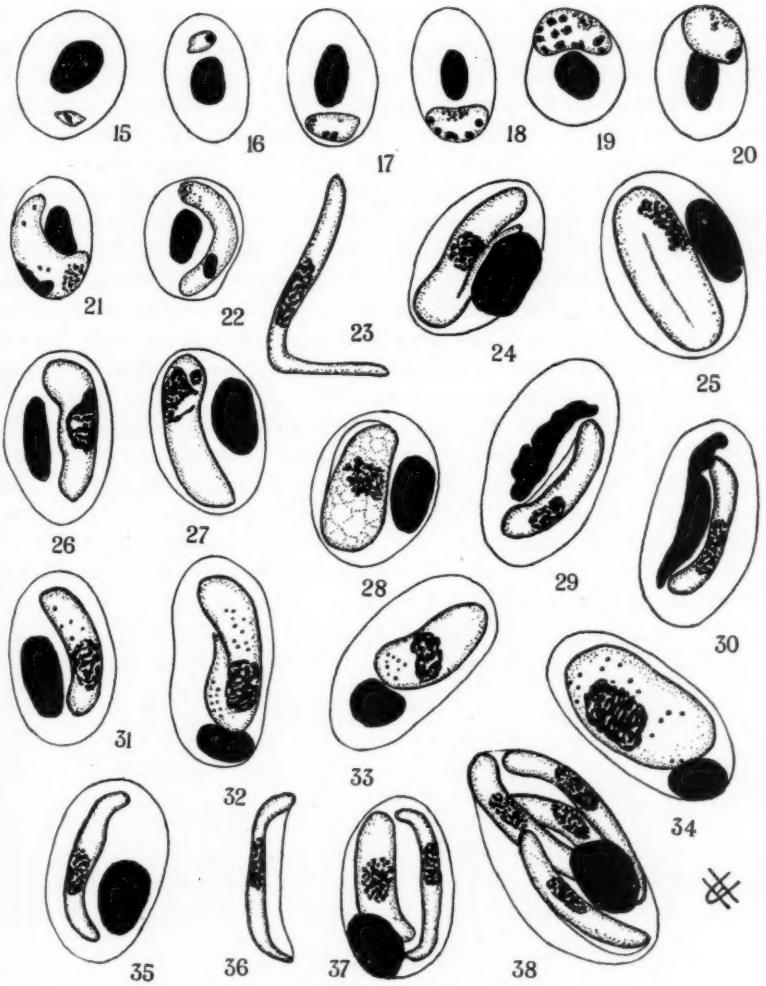
Haemogregarines of snakes.

Simocephalus butleri. One specimen of this snake, called "vubirizi" by the natives, was obtained near Entebbe. It was fairly heavily infected with haemogregarines. The majority of these (Fig. 26) are thick, bean-shaped, measuring $15–17 \times 3{\cdot}8–4{\cdot}7\mu$. The host cell is unaltered. As this is the first record of the parasite in this snake, I propose for it the name *Haemogregarina vubirizi* n.sp.

Boaedon lineatus. This snake is one of the commonest near Entebbe. Out of twelve examined one was found to be infected with a fairly large number of haemogregarines. These have not hitherto been recorded for this snake. Some of the parasites are bean-shaped, measuring $17 \times 3{\cdot}8–4{\cdot}7\mu$ (Fig. 27); others are broad forms with vacuolated cytoplasm, measuring about $15{\cdot}2 \times 6{\cdot}6\mu$ (Fig. 28). It is proposed to name this parasite *Haemogregarina musotae* n.sp.[1]

Crotaphopeltis (= *Leptodeira*) *hotamboeia.* Five of these snakes, obtained near Entebbe, were examined, and in one of them haemogregarines were found. A haemogregarine was previously recorded for this snake by Plimmer (1916) from West Africa, but not named. The present case is particularly interesting, on account of the exceptionally heavy infection. The snake was kept in the laboratory for about 4 days. At the end of this period it appeared to be moribund and was dissected. About 50 per cent. of its red cells were found to harbour haemogregarines, multiple infections being predominant, with from two to six parasites in each cell (Figs. 35–38), while some parasites were extracellular, enclosed by their capsule only (Fig. 36). Many of the host cells were

[1] From the Luganda "musota" = snake.



Figs. 15–38. Haemococcidia.

15–22, *Plasmodium pitmani* n.sp. from skinks (15, 16, trophozoites; 17–19, schizonts, 20, young gametocyte; 21, 22, mature gametocytes). 23–25, *Haemogregarina hyperolii* n.sp. from frog. 26, *H. vubirizi* n.sp. from snake. 27, 28, *H. musotae* n.sp. from snake. 29–31, *H. enswerae* n.sp. from cobra. 32–34, *H. sternothaeri* from tortoise. 35–38, *H. crotaphopeltis* n.sp. from snake. (× 1500.)

enormously distended and deformed, the nucleus having been in some cases extruded.

It is possible that the state in which the snake was found is attributable to this exceptionally heavy infection, since others kept under identical conditions suffered no inconvenience.

The haemogregarine appears mainly in the form of slender vermicules measuring about $20 \times 2\mu$, but bean-shaped forms were also seen. For this parasite the name *Haemogregarina crotaphopeltis* n.sp. is proposed.

Naja melanoleuca. Three specimens of this cobra were obtained from Entebbe and Kampala. They were all infected with a haemogregarine, not previously reported for this host. The majority of the parasites are slender vermicules, measuring $19 \times 3\mu$ (Figs. 29, 30); others are bean-shaped, measuring $15 \times 3{\cdot}8\mu$ (Fig. 31). The red cells containing the slender forms are dehaemoglobinised and their nuclei are drawn out in length and deformed, whereas those harbouring the broad forms are apparently unaltered. I propose to name this parasite *Haemogregarina enswerae* n.sp.[1]

Python sebae. Numerous haemogregarines (*H. sebai*) were found in the blood of one python. An interesting observation was made on the vitality of these parasites *in vitro.* Blood cultures were made in Nöller's medium and examined during 9 days. It was found that the haemogregarines had escaped from the blood cells and from their capsules and were free in the medium. When observed between slide and coverslip they were seen to be motile up to the last day of examination.

(*c*) *Chelonia.*

A haemogregarine from a tortoise.

Two tortoises, *Sternothaerus sinuatus,* from the shore of Lake Victoria, near Entebbe, were found to be infected with a haemogregarine. The majority of the parasites (Figs. 32–34) are doubled up and measure about 26μ in length, in others the limbs appear to be fused.

This parasite agrees closely with that described by Wenyon (1909) from *S. adansoni.* Similar forms were described from *S. derbianus* and *S. niger.* The former bears the name *Haemogregarina sternothaeri* França 1910. It is probable that all the haemogregarines from *Sternothaerus* spp. belong to this species.

(*d*) *Crocodilia.*

The life cycle of the crocodile haemogregarine.

I had the opportunity of examining the blood of a fair number of crocodiles (*Crocodilus niloticus*) in connection with my investigations on the life cycle of *Trypanosoma grayi.* Every one of them was infected with haemogregarines. This parasite was first described in detail by Thiroux (1910), who named it *Haemogregarina pettiti.* It was noted earlier by Minchin, Gray and Tulloch (1906) and by Wenyon (1909), but no description was given. The same parasite

[1] From the Luganda "enswera" = cobra.

was seen by Minchin (1910) and Leger, M. and A. (1914). All these records referred to the stages in the blood exclusively.

In a second paper Thiroux (1913) described what he took to be the schizogony and sporogony of this parasite in the liver of the crocodile. There can be no doubt, however, that the stages seen by him did not belong to the haemogregarine, but actually represented the sporogony of a coccidian of the genus *Eimeria*, which apparently completes its development in this organ. This interpretation was originally suggested by Mesnil (1913). It is thus seen that the further development of this haemogregarine in the crocodile has hitherto remained unknown, nor has its intermediate host been discovered. In the present study sufficient data were obtained to complete the life history of this parasite.

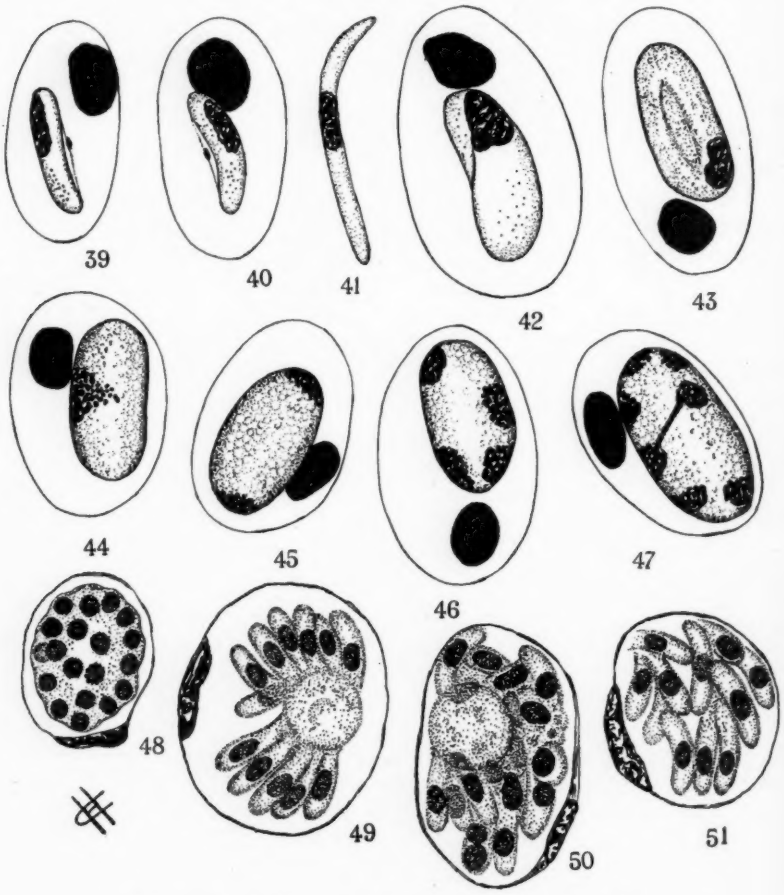
The crocodiles examined were killed or captured in Lake Victoria and ranged from young individuals, not more than 1 month old, to full-grown adults. Though all were infected, the parasites were very scanty in their blood. The blood forms of the haemogregarine are similar to those described by previous observers. The stages most frequently encountered are slender, encapsulated sausage-shaped forms with an elongated nucleus, measuring about 11·5μ in length (Figs. 39, 40). A few longer vermicular forms (Fig. 41), about 24·7μ long, are found free in the blood. Some of the parasites are fairly broad and doubled up (Fig. 42). The host cells harbouring the latter form appear to be hypertrophied.

Smears and sections of the various organs of the crocodile were examined for other stages of development of the parasite. It was established that the asexual multiplication occurred in the liver exclusively. The initial stages of schizogony take place in the red cells. The limbs of the doubled up parasite apparently first fuse together (Fig. 43), the body becomes plumper and the cytoplasm vacuolated, while the nucleus takes up a more central position (Fig. 44). Later the nucleus undergoes a series of successive divisions, while the body continues to increase in size and assumes an irregular ellipsoid shape (Figs. 45–47). The host cells in which schizogony is proceeding are enlarged. The early stages of schizogony were found in the liver smears of a young crocodile: their further development was observed in sections of the liver of an adult individual.

It would appear that when schizogony has proceeded for some time the red cells enclosing the parasite rupture and the schizonts are taken up by the Kupffer cells, for the final stages of schizogony are enclosed by a thin membrane with a flattened nucleus, typical of the Kupffer cells, lying at one side. These mature schizonts occur exclusively in the lumen of the narrow parts of the liver sinusoids which, as is known, are lined by Kupffer cells, and have never been seen inside the liver cells. The mature schizont (Fig. 48) is a rounded body containing over thirty nuclei arranged on the periphery. As the merozoites are budded off, part of the cytoplasm of the schizont is left over as a globular residual body (Figs. 49, 50). The merozoites when fully formed (Fig. 51) are

similar in size and shape to the slender forms occurring in the blood of the crocodile (Figs. 39, 40), to which they presumably give rise.

The sexual cycle of development, or sporogony, of this parasite, was discovered by me quite accidentally. In the course of experiments on the transmission of the crocodile trypanosome by the tsetse fly (*Glossina palpalis*)



Figs. 39–51. Development of *Hepatozoon pettiti* in the crocodile.
39–43, blood forms. 44–51, stages of schizogony in the liver. (× 2000.)

laboratory-bred ("clean") flies were fed on a crocodile infected with *Haemogregarina pettiti* and *Trypanosoma grayi*. In one of these flies, dissected on the twentieth day after feeding on the crocodile, large cyst-like globular bodies full of smaller cysts were found in the body cavity. The small cysts contained numerous vermicular organisms which were motile when released. These

structures appeared to be identical with those described by Chatton and Roubaud, and by Macfie.

Chatton and Roubaud (1913) found cysts of the same type in four out of 465 "wild" *G. palpalis* examined in West Africa. In these cysts all the stages of a development interpreted as the sporogony of a haemogregarine of the *Hepatozoon* type were discovered. Each cyst, or oocyst, contained about 100 sporocysts in which sporozoites are produced. The whole development is described in detail and illustrated by excellent figures. The authors believed that the stages seen by them belonged to a haemogregarine of either the monitor, crocodile, or some small lizard on which the tsetse flies had fed.

Similar bodies were observed by Macfie (1916) in the abdominal cavity of two *G. palpalis* from the Gold Coast. As in the preceding case the sporogony was followed up to the formation of sporozoites. Macfie also regarded these cysts as representing the developmental stages of a haemogregarine, and suspected either a lizard (*Agama colonorum*) or the crocodile to be the vertebrate host. As a lizard was fed on the cysts, but failed to acquire an infection, it seemed more probable that the crocodile was the host. This appeared to be all the more likely since the flies in which the haemogregarine cysts were found, both by Macfie and by Chatton and Roubaud, were also infected with *T. grayi*.

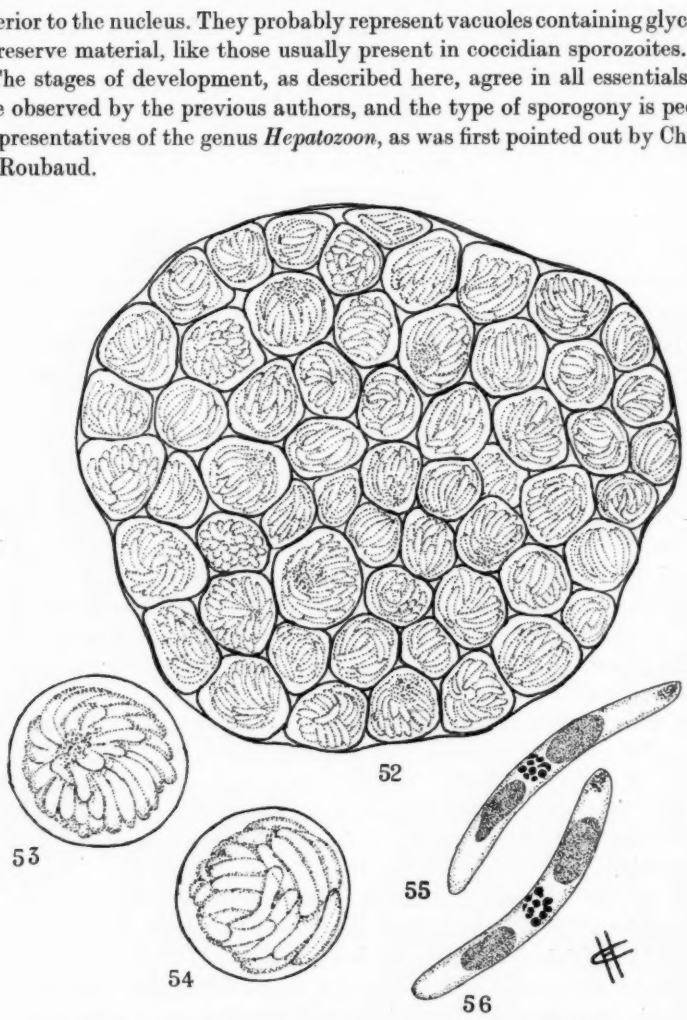
The case described in this paper settles the question beyond all doubt, as the fly in which the oocysts were found could not have acquired its infection from any other source but the crocodile on which it had fed. After the infective meal the fly was kept alive on sheep's blood.

As I did not at first fully realise the nature of these bodies I did not fix any of them for sections, but made dry and wet fixed smears of the abdominal contents of the fly, stained with Giemsa's solution and Heidenhain's iron haematoxylin respectively. The results were not quite satisfactory, as the oocysts were flattened and the sporozoites within the sporocysts failed to take up the stain.

The oocysts seen by me (Fig. 52) have an irregular rounded outline and measure from 258μ to 300μ across in one direction, and about 237μ in another direction, but originally, when seen fresh, they were spherical, like the cysts described by the preceding observers. By examining different planes it could be estimated that the oocyst contains over 100 sporocysts. Isolated sporocysts (Figs. 53, 54) are spherical, the majority being 38μ in diameter, others up to 53μ. The number of sporozoites in each sporocyst could not be ascertained. Within the sporocyst the sporozoites appear to be arranged in the form of a bunch of bananas around a residual body (Fig. 53), but in some cases they are distributed irregularly (Fig. 54). In preparations stained with iron haematoxylin the isolated sporozoites measure about 18μ in length (Figs. 55, 56). The body can be described as banana-shaped. The nucleus is composed of an accumulation of coarse chromatin granules and is situated more or less centrally. There are two oval, darkly staining bodies, one anterior to and the other

posterior to the nucleus. They probably represent vacuoles containing glycogen-like reserve material, like those usually present in coccidian sporozoites.

The stages of development, as described here, agree in all essentials with those observed by the previous authors, and the type of sporogony is peculiar to representatives of the genus *Hepatozoon*, as was first pointed out by Chatton and Roubaud.



Figs. 52–56. Development of *Hepatozoon pettiti* in the tsetse fly. 52, oocyst with ripe sporocysts (× 350). 53, 54, sporocysts with sporozoites (× 650). 55, 56, isolated sporozoites (× 2500).

These haemogregarines are known chiefly as parasites of the leucocytes and erythrocytes of mammals. Their occurrence in birds was first established by myself (Hoare, 1924), while the present record extends the distribution of *Hepatozoon* to reptiles.

This is the first instance in which a dipteran insect has been proved to be the intermediate host of a haemogregarine. In the light of the observations

made on the transmission of *T. grayi* to the crocodile (Hoare, 1929) it is obvious that the transmission of the haemogregarine is effected in a similar manner, *i.e. per os*, as in other members of the genus *Hepatozoon*. It is well known that the crocodile habitually spends part of the day on land, lying motionless with its mouth wide open. If a tsetse fly settles in the mouth of the crocodile to feed, as it does under experimental conditions, the crocodile, irritated by the bites, may snap its jaws and crush the fly, thus liberating the cysts of the haemogregarine in the buccal cavity. In accordance with the facts established above, the haemogregarine of the crocodile should be known henceforth as *Hepatozoon pettiti* (Thiroux 1910) emend.

IV. AVES.

Representatives of the following genera and species of birds were examined for blood parasites with negative results: *Camaroptera* sp., *Corvinella* sp., *Halcyon* sp., *Hyphantornis* sp., *Lamprocolius* sp., *Lophoaëtus occipitalis*, *Macronyx* sp., *Milvus migrans*, *Parasoma* sp., *Phalacrocorax a. africanus*, *P. carbo lugubris*, *Prinia* sp., *Sterna albigena*, *Streptopelia* sp., *Tschagra* sp., *Turtur* sp., *Vinago* sp.

A Plasmodium *of Saxicola.*

One *Saxicola salex saxilaris*, from Bugerere district, was found to be infected with a *Plasmodium* hitherto unrecorded for this bird. Typical schizonts and gametocytes were seen in the blood.

V. MAMMALIA.

The blood of the following mammals was examined with negative results: *Chaerephon limbatus* (about 100 individuals), *Epomophorus* sp., *Rhinolophus* sp., *Manis tricuspis*, squirrels, mongooses, jackal, *Bos bubalus caffer*, *Cobus adenota thomasi*, *Cobus ellipsiprymnus*, *Guevei monticola*, *Redunca redunca*, *Colobus* sp., *Cercocebus* sp.

A trypanosome of the potto.

Trypanosomes were found in the blood of a potto, *Perodicticus ibeanus* (Nycticebidae), caught near Entebbe and kept in captivity. The parasites were extremely scanty, only six specimens having been seen in over a dozen films.

Reichenow (1917) was the first to record a trypanosome in a West African potto, *Perodicticus* sp. It was named *Trypanosoma lewisi primatum*, but later (Reichenow, 1928) the name was changed to "*T. pterodictici*." As this is obviously a *lapsus calami*, it should be altered to *T. perodictici*.

My preparations show two types of trypanosomes. One is similar to *T. lewisi* and to *T. perodictici*, and measures from 36·6μ to 41·3μ in length, including the free flagellum (Figs. 5, 6). The kinetoplast is very near the posterior end of the body, and there is a fairly long free flagellum. The other form is considerably larger, measuring 61·3μ in length, with the kinetoplast in close proximity to the nucleus, and a frilled undulating membrane (Fig. 7). It

List of blood parasites collected in Uganda.

Note: The second column includes the following: new forms described by me in this paper and elsewhere; known parasites recorded from new hosts; and parasites on which new observations have been made. The third column contains parasites originally described by previous workers, and found by me in the same host.

No.	Hosts	Protozoa: New	Protozoa: Known
	Pisces		
1.	*Haplochromis nubilus*	*Dactylosoma mariae* Hoare 1930 *Trypanosoma mukasai* n.sp.	
2.	*H. cinereus*	*D. mariae* Hoare 1930 *T. mukasai* n.sp.	
3.	*H. humilior*	*T. mukasai* n.sp.	
4.	*H.* sp.	*D. mariae* Hoare 1930	
5.	*H. serranus*	*T. mukasai* n.sp. *D. mariae* Hoare 1930	
	Amphibia		
6.	*Hyperolius* sp.	*Haemogregarina hyperolii* n.sp. *Trypanosoma* (? *rotatorium*)	
	Reptilia		
	(*a*) Lacertilia		
7.	*Mabuya striata*	*Plasmodium pitmani* n.sp.	*Trypanosoma martini* Bouet 1909
8.	*M. maculilabris*		
9.	*Varanus niloticus*		*Haemogregarina varani* Laveran 1905
	(*b*) Ophidia		
10.	*Bitis arietans*		*H. arietans* Fantham 1925
11.	*B. gabonica*		*H. dogieli* Hoare 1921
12.	*Boaedon lineatus*	*Haemogregarina musotae* n.sp.	
13.	*Chlorophis emini*		*H.* sp. Wenyon 1909
14.	*Crotaphopeltis hotamboeia*	*H. crotaphopeltis* n.sp.	
15.	*Naja melanoleuca*	*H. enswerae* n.sp.	
16.	*Psammophis sibilans*		*H. brendae* Sambon and Seligmann 1907
17.	*Python sebae*		*H. sebai* Laveran and Pettit 1909
18.	*Simocephalus butleri*	*H. vubirizi* n.sp.	
	(*c*) Crocodilia		
19.	*Crocodilus niloticus*	*Hepatozoon pettiti* emend.	*Trypanosoma grayi* Novy 1906
	(*d*) Chelonia		
20.	*Sternothaerus sinuatus*	*Haemogregarina sternothaeri* França 1910	
	Aves		
21.	*Francolinus levaillanti*		*Leucocytozoon* sp. Plimmer 1912
22.	*Lissotis melanogaster*		*Haemoproteus* sp. Todd and Wolbach 1912
23.	*Numida meleagris*		*Leucocytozoon* sp. Kerandel 1909
24.	*Saxicola salex*	*Plasmodium* sp.	
	Mammalia		
25.	*Cercopithecus pygerythrus*		*Plasmodium kochi* Laveran 1899
26.	*Crocidura bicolor*	*Babesia crocidurae* Hoare 1930	
27.	*Limnotragus spekei*		*Trypanosoma tragelaphi* Kinghorn *et al.* 1913 *T. ingens* Bruce *et al.* 1909
28.	*Praomys jacksoni*	*Trypanosoma lewisi* Kent 1880	
29.	*Perodicticus ibeanus*	*T. perodictici* Reichenow 1928	
30.	*Rattus rattus*		*T. lewisi* Kent 1880
31.	*Tragelaphus scriptus*		*T. tragelaphi* Kinghorn *et al.* 1913 *T. uniforme* Bruce *et al.* 1911

closely resembles the trypanosomes of some land reptiles. In view of the scanty number of parasites found in the potto, it is impossible to decide whether these two forms belong to one species, or to two distinct ones.

The two specimens of trypanosomes depicted by Reichenow (1928) measure about 22μ and 25μ in length, as calculated by me from the figures, and show no free flagellum. It is probable, however, that this structure was overlooked, since Reichenow's figures were drawn from a thick film. The smaller dimensions of his trypanosome would thus be accounted for. Until more is known about these parasites it is best to regard the one from the East African potto as *T. perodictici* Reichenow 1928.

A trypanosome of a rat.

A trypanosome similar to *T. lewisi* was found in a local rat, *Praomys jacksoni*, from which this parasite has not previously been recorded (Figs. 10–14). Small forms of this trypanosome measure 14·6–17·3μ in length, while larger forms are over 36μ long, the average length being about 26μ. Multiple division, similar to that in the typical rat trypanosome, was observed. It is probable that the intermediate host of this trypanosome is *Xenopsylla cheopis*, as a number of these fleas were found on the rat.

SUMMARY.

This paper contains a report on a collection of parasitic protozoa from the blood of some vertebrate animals of Uganda.

Seven new species and a number of parasites recorded for new hosts are described. New observations on some known parasites are also recorded.

An account is given of the life history of the crocodile haemogregarine. It is shown that the schizogony of *Hepatozoon pettiti* (*nomen novum* for *Haemogregarina pettiti*) occurs in the liver of the crocodile, while the sporogony takes place in *Glossina palpalis*, its intermediate host.

A list of all the blood parasites found, together with their hosts, is given.

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ENTAMOEBA AULASTOMI NÖLLER.

CULTIVATION, MORPHOLOGY, AND METHOD OF DIVISION; AND CULTIVATION OF *HEXAMITA* SP.

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From the Molteno Institute, Cambridge.

(With Plate XI, containing 26 Figures.)

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I. Cultivation of *E. aulastomi* and *Hexamita* sp.

Entamoeba aulastomi was discovered and described by Nöller (1912), who found it in large numbers in the hind gut of well-nourished horse-leeches (*Aulastomum gulo* = *Haemopis sanguisuga*, *vide* Harding (1910)).

Drbohlav (1925) cultivated *E. aulastomi* in Boeck's medium, blood agar, ordinary agar, and starch agar, covered with ovomucoid liquid. Although he varied the NaCl content from 0·6 to 0·3 per cent. he was unable to obtain a rich growth. The amoebae never formed cysts in his cultures. He found the optimum temperature to be 20° C.

On 19. vi. 1931 a horse-leech (*Haemopis sanguisuga*) was caught in a ditch in Cambridge. A culture was made from the contents expressed from the hind gut. The medium used was inactivated horse-serum diluted 1 : 10 in 0·5 NaCl solution and a little sterile solid rice-starch was added to each tube. Within 5 days there appeared an extraordinarily thick culture of *E. aulastomi* mixed with a large flagellate of the genus *Hexamita* (= *Octomitus* Prowazek (1904) and Dobell (1909)). *Hexamita* multiplied more rapidly than *E. aulastomi*, but died out of each culture a week or 10 days before the amoebae. Thus "pure" cultures (*i.e.* cultures containing only one species of protozoan but with mixed bacteria) of *E. aulastomi* were obtained. As growth in this wholly liquid medium was variable, the amoebae were later grown upon a medium consisting of an inspissated horse-serum slope covered with the liquid medium described above and with the sterile rice-starch added. (A detailed account

of their work on media of this type and of the necessary technique was published by Dobell and Laidlaw (1926).) Cultures of *E. aulastomi* needed subcultivating every 14 to 20 days when grown at room temperature (18–22° C.). After six months the strain is still producing luxuriant cultures. The amoebae ingest much starch as well as bacteria. Enormous crops of cysts were, and still are, produced in the cultures (p. 228) on the 9th to the 14th day.

The strain of *Hexamita* mixed with *E. aulastomi* was kept alive upon this part-solid part-liquid medium by subcultivating every 7 days. This flagellate, although without a mouth, ingests much starch. The cuticle of the posterior end appears to be sticky, for the starch grains and bacteria adhere to it and are gradually engulfed. In September Mr Clifford Dobell, F.R.S., very kindly offered to take care of my cultures for three weeks whilst I went on holiday. During this time he obtained a pure culture (in the sense defined above) of *Hexamita* by picking off a film from the surface of a culture and putting it into a tube of medium. In the film were a few flagellates but no amoebae. I am greatly indebted to him both for his care of my cultures and for isolating for me a strain of *Hexamita*. This flagellate is still producing excellent cultures and is subcultivated every 6th or 7th day. No cysts have been found in these cultures.

My preparations of *E. aulastomi* were fixed for 10 min. to half an hour with Schaudinn's solution, with acetic acid varying in amount from 1 to 5 per cent. The greater concentration was suitable for the amoebae, but good preparations of the cysts were only obtained when the acetic acid present was not less than 1 per cent. and not greater than 2·5 per cent. The cysts were exceedingly difficult to fix satisfactorily, and preparations of them needed to be hardened in 90 per cent. alcohol for at least 14 days before staining. Good preparations of the amoebae were also obtained after fixation with Bouin's fluid, but this gave poor results with the cysts. All preparations were stained with iron haematoxylin using the mordant and the stain for 24 hours.

II. Morphology of *E. aulastomi*.

E. aulastomi is a small amoeba 10–18μ when rounded. Nöller gives its measurements as 20–32μ, but only rarely have individuals occurred in cultures measuring more than 18μ. The amoeba is very active at room temperature and moves by the thrusting out of a single pseudopod in the direction of progression. There is little differentiation of ecto- from endoplasm, and the clear, blade-like pseudopodia characterising *E. histolytica* are lacking in this species. The cytoplasm is frequently so full of starch grains that the nucleus (Fig. 2) is distorted. The single nucleus is spherical and is bounded by a delicate membrane. On the inner surface of this membrane blocks of chromatin are deposited giving, in optical section, a beaded appearance. A small central karyosome is present surrounded by a small clear zone. The karyosome is generally spherical (Fig. 1), but may (Fig. 2) be four-lobed. There are no extra-karyosomic granules. The nucleus is 3–5μ in diameter.

This description of the nucleus differs in some points from that of Nöller (1912). He observed the chromatin blocks on the inner side of the nuclear membrane, but described the karyosome as finely granular. The karyosome, according to his description and figures, was much larger and more diffuse than I have found it except in forms about to divide, and he also described a centriole in its centre, a structure which I have never seen. His measurements of the diameter of the nucleus varied from 1·5 to 10μ. Drbohlav (1925) did not describe the nucleus.

III. Division of *E. aulastomi*.

In the early stages of division the karyosome increases considerably in size and appears (Figs. 3 and 4) to be composed of several granules. The clear zone surrounding it disappears. The beaded appearance of the inner surface of the nuclear membrane is lost and the amount of chromatin there decreases. The nucleus becomes elongated (Fig. 5), and in the centre a collection of chromatin granules varying slightly in size are found. It has been impossible to count them. There is no sign of spindle formation such as appears in the dividing nucleus of *E. histolytica* (Dobell, 1919; Dobell and O'Connor, 1921; and Dobell, 1928). The nucleus elongates further (Fig. 6) and begins to constrict in the middle. The granules in the middle of the nucleus appear variable in size and number and frequently stain lightly. So far as could be ascertained an equal number of them move towards either pole of the constricting nucleus. The nucleus (Fig. 7) becomes completely constricted. The two halves then pull apart (Fig. 8) though they are still joined by a delicate strand formed by the constricted nuclear membrane. The body of the amoeba begins to elongate and the strand connecting the daughter nuclei breaks through. The chromatin blocks on the inner side of the nuclear membrane reappear and the granules in the centre of the nucleus become less numerous. The vestige of the connecting strand may be absorbed before (Fig. 11) or not till after (Figs. 9 and 10) the body divides. The irregular granules in the centre of the nucleus gradually become concentrated to form the karyosome of the resting phase. In some individuals division of the body evidently does not occur and binucleate amoebae (Fig. 12) are formed which are usually larger than normal individuals. The proportion of these in a culture is small. Binucleate *E. aulastomi* were observed and figured by Nöller (1912). Large multinucleate amoebae such as I have seen in cultures of *E. ranarum* and sometimes in cultures of *E. histolytica* I have never seen in *E. aulastomi*. The greatest number of dividing amoebae are found on the 4th or 5th day from subcultivation.

The method of division in the trophic stage of *E. aulastomi* is very simple and direct. It is impossible to compare it with division in other species of entamoebae, with the exception of *E. histolytica*, as little is known of their method of division. After many years of laborious study upon *E. histolytica* Dobell (1928, p. 378) says of the division of its trophic stage: "At present I believe that the division is a mitosis of a peculiar kind, and that the chromo-

some number is probably constant: but what it really is I do not know. In different specimens I have counted, quite clearly, all numbers from five to eight. I am inclined to think that the latter figure is probably the real number." But the division of the tropic phase of *E. aulastomi* differs essentially from that of *E. histolytica*.

IV. Method of cyst formation of *E. aulastomi*.

Cysts of *E. aulastomi* appeared in the cultures from the 9th to the 14th day. Just before the cyst formation begins the majority of the amoebae begin to stain darkly with iodine. When fixed and stained with iron haematoxylin the precystic amoebae (Fig. 13) contain many intensely staining rod-like bodies. These appear to be the first stage in the formation of the chromatoid bodies. They vary considerably in size but are never the large block-like bodies present in the cysts. No evidence of a nuclear origin could be deduced. They seemed to appear as minute bodies in the cytoplasm. Their presence seemed universal in the precystic amoebae. Dobell (1909) found chromatoids (and named them) in the precystic stage of *E. ranarum*. In the precystic stage of *E. histolytica* he (1928) says they occur occasionally.

In all cultures the cysts are very numerous, occurring frequently in great masses. The majority of the cysts are spherical but a few are oval or egg-shaped. They range in size between 8 and 13 μ, but the majority measure 9 to 11 μ. Uninucleate cysts (Fig. 14) contain large glycogen vacuoles and the nucleus and chromatoids are frequently flattened against the cyst wall (Fig. 15). A thick outer cyst wall is formed and the contents of the cyst is limited by an inner wall. The distance between the inner and outer wall varies. In fixed preparations this variation may be intensified by shrinkage, but it is also demonstrable in the living cyst and is very marked in cysts stained with iodine. The nucleus in the uninucleate cyst varies considerably in size and sometimes is elongated through the pressure of the glycogen vacuole. The chromatoids are numerous and massive. During the uninucleate stage the glycogen is absorbed. When the nucleus is ready to divide, the karyosome (Fig. 17) increases in size and appears to give rise to a number of granules as in division in the trophic stage. The chromatin blocks on the nuclear membrane become less accentuated. The nuclear membrane becomes pulled out at opposite poles and a spindle-shaped structure is formed (Fig. 18), sharply pointed at the poles. The nuclear membrane bounding the spindle still shows traces of the chromatin blocks. Two or three delicate strands bearing minute and irregularly placed granules of chromatin run through the centre of the spindle, but, though I have seen many such spindles, I have never found anything approaching chromosomes or an equatorial plate. The spindle elongates (Fig. 19) so greatly that the ends become bent. Fine chromatin blocks are still visible on the elongated nuclear membrane. The spindle constricts (Fig. 20) in the centre, and two daughter nuclei (Fig. 21) arise from the two halves. Whereas the number of uninucleate cysts in a culture just

beginning to encyst, and of quadrinucleate cysts in the same culture 24–36 hours later, is very great, the binucleate cysts are never very numerous; and although many preparations have been searched, with the exception of a few examples showing increase in size and formation of granules in the karyosomes (Fig. 22), no stages of division of binucleate cysts have been found. Although it is easy to make good preparations of uninucleate cysts, it is very difficult to obtain good results with binucleate and quadrinucleate cysts. Trinucleate cysts have not been found. In the majority of quadrinucleate cysts the nuclei are equal (Fig. 25) in size, but occasionally cysts are found (Fig. 23) containing one large and three small nuclei. How this condition arises and whether such cysts are capable of hatching has not been discovered. A few octonucleate cysts (Fig. 24) have been found. Whether these result from an extra nuclear division or the encysting of a binucleate amoeba is not known.

As the quadrinucleate cyst increases in age the chromatoids gradually disappear. When the cyst is ripe for hatching (Fig. 26) no chromatoids are present, the cytoplasm is slightly vacuolated and the content of the cyst completely fills the outer cyst wall. A small quadrinucleate amoeba, having its nuclei clumped together, as in the recently excysted amoeba of *E. histolytica* (Dobell, 1928), hatches from the cyst. I hope to describe the metacystic development in a later paper. The whole course of development from the formation of the uninucleate cyst to the mature quadrinucleate cyst seems to vary from 24 to 56 hours. If a culture containing mature cysts is not subcultivated, a few of the cysts hatch in the medium, but the majority become vacuolated and die.

Nöller (1912) observed cysts of *E. aulastomi* with nuclei varying in number from one to eight, but he concluded that the quadrinucleate was the normal mature cyst.

Though quadrinucleate and with chromatoids like the cysts of *E. histolytica* the cysts of *E. aulastomi* differ in having a double cyst wall. The strain of *E. ranarum* described by Dobell (1909) is much larger than this strain of *E. aulastomi*, but Sanders (1931) has described the metacystic development of a strain of *E. ranarum* similar in size to my *E. aulastomi*. As she has not described division in the trophic stage or in the cyst, it is not possible to compare these stages.

Few entamoebae from invertebrate hosts are known. Mackinnon (1914) described an amoeba which she called *Löschia hartmanni* from the intestine of the crane-fly larva. The amoeboid stage had the nuclear structure characteristic of entamoebae, but the cysts had remarkably thick walls and contained at least ten nuclei. There were no chromatoids. She believed that encystment was preceded by a copulation of gametes. Brug (1922) has described an entamoeba with a nucleus like *E. histolytica* from *Belostoma indicum*, but he found no cysts. Lucas (1927) described an entamoeba (*E. thompsoni*) from *Blatta orientalis*. The mature cysts were quadrinucleate and contained no chromatoids. I have, in the course of the last four years, examined the gut of

many cockroaches and have found cysts identical with those of Lucas's description. It is impossible with our present scanty knowledge of them to deduce any relationship between entamoebae parasitic in invertebrate hosts or to compare them with the entamoebae from vertebrate hosts.

V. Some experiments upon cultures of *E. aulastomi*.

(*a*) *The effects of temperature.*

Cultures of *E. aulastomi*, if submitted to a temperature of + 2° C., will live 3–9 weeks and grow well when subcultivated into new medium if kept at room temperature. But although the amoebae survive this low temperature division must be suspended, as new cultures containing few amoebae and submitted to this low temperature do not multiply.

Rich cultures of *E. aulastomi* were placed in an incubator at 30° C. The cultures grew very poorly, and though subcultivated on the 5th day (when growth should have been at the maximum) they died out in the 2nd subculture. This experiment was repeated five times, but no strain survived. If rich cultures were subjected to a constant temperature of 37° C. they died within 24 hours and subcultures made from them and kept at room temperature were negative. Cysts were not less resistant. This lethal action of heat upon *E. aulastomi* was noted by Drbohlav (1925), who found that entamoebae died rapidly at 30 and 37° C. *E. aulastomi* differed in this quality from the strain of *E. ranarum* cultivated by Sanders (1931). She was able to keep cultures alive at 37° C. for 2 weeks, though they gradually decreased in number. She also found that by incubating cysts of *E. ranarum* for 4–10 hours at 37° C. the absorption of the glycogen and chromatoid bodies was accelerated. Cysts of *E. aulastomi* treated thus never hatched.

Low temperature (+ 4–0° C.) had no effect in inducing the formation of cysts in *E. aulastomi*.

(*b*) *Action of N/20 hydrochloric acid.*

In order to study the metacystic development of *E. aulastomi* it was necessary to find a method of killing off all the free amoebae in an encysting culture, whilst preserving the cysts unharmed. In his series of brilliant papers upon *E. histolytica* Dobell (1927) made many careful experiments upon the action of *N*/20 HCl upon that amoeba and evolved a method by which he was able to work out the metacystic development in great detail. He found that, whereas at room temperature the majority of the amoeboid stage of *E. histolytica* was killed off in a few minutes, a few survived up to 20 min., but the cysts were unharmed by treatment with *N*/20 HCl for 30 min.

In order to estimate the length of time necessary to kill off all the amoebae present in a culture of *E. aulastomi* it was necessary to be certain that no cysts were present. A 4-day-old culture was examined very carefully and no cysts were found. It was subcultivated and the original culture kept. Cysts

were found in the latter on the 10th day. The subculture was examined and found negative for cysts and subcultivated on the 4th day. This was performed for a series of four subcultures without finding any cysts on the 4th day—the cysts appearing on the 9th day or later. Material obtained as a result of this series of subcultivations on the 4th day was used for the experiments with *N*/20 HCl. Repeated experiments showed that the majority of the amoebae were killed by *N*/20 HCl in a few minutes and that none survived for 10 min. The results were based upon cultures made from the neutralised material, and the cultures so made were always negative when examined on the 5th and 9th day. In a few experiments subcultures were made from these negative cultures and were themselves negative. Cysts, however, treated with this concentration for 10 min. hatched well, though if treated for longer than 15 min. almost all were killed. They appeared much more susceptible to *N*/20 HCl than were the cysts of *E. histolytica*.

Sanders (1931) found that cysts of *E. ranarum* required to be stored for 7–10 days in order that their reserves might be absorbed and the cysts ready to hatch. No such period of storage is necessary in *E. aulastomi*.

VI. SUMMARY.

1. *Entamoeba aulastomi* and *Hexamita* sp. from *Haemopis sanguisuga* have been cultivated in inactivated horse-serum diluted 1 : 10 in 0·5 NaCl solution + solid sterile rice-starch, and upon an inspissated horse-serum slope covered with the liquid medium + sterile rice-starch. Cysts of *E. aulastomi* appeared on or after the 9th day from subcultivation, but cysts of *Hexamita* have not appeared in the cultures.

2. The amoeboid phase of *E. aulastomi* divides by a simple direct method, without the formation of a spindle. There is no equatorial plate. It was not possible to count the number of chromatin granules.

3. The mature cyst is quadrinucleate, though occasional octonucleate cysts are found. Chromatoids are present but are absorbed before hatching. A large glycogen vacuole is present in the uninucleate cyst. The nucleus divides by the formation of a delicate spindle but no chromosomes were found.

4. *E. aulastomi* are not harmed by being kept at 2° C. but they die out in the 2nd subculture if kept at 30° C. and within 24 hours if kept at 37° C. The cysts also are killed at 37° C.

5. If treated with *N*/20 HCl all amoebae are killed in less than 10 min. but the cysts are unharmed.

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DESCRIPTION OF PLATE XI

All figures were drawn with an Abbé camera lucida under a $\frac{1}{12}$ in. A fluorite oil-immersion lens (Leitz) at an approximate table magnification of 3300 diameters.

Entamoeba aulastomi.

1. Amoeboid stage containing ingested starch and bacteria.
2. The same.
3 and 4. Nucleus preparing for division, showing increase in size of karyosome and formation of granules from it.
5. Elongation of the nucleus.
6. Nucleus beginning to constrict.
7. Constriction further advanced.
8. Daughter nuclei drawing apart.
9. Daughter nuclei separated but each bearing the vestige of the drawn-out nuclear membrane.
10. Daughter amoeba. Nucleus not yet completely reorganised.
11. Division of daughter nuclei completed, and body of amoeba showing constriction.
12. Binucleate amoeba.
13. Precystic amoeba containing chromatoids.
14. Living cyst stained with iodine.
15. Uninucleate cyst containing large glycogen vacuole.
16. Uninucleate cyst with glycogen vacuole decreasing in size. Large chromatoids present.
17. Uninucleate cyst with nucleus preparing for division.
18. Formation of spindle from nucleus.
19. The same further advanced.
20. The same constricting.
21. Binucleate cyst.
22. Binucleate cyst with nuclei preparing for division.
23. Quadrinucleate cyst with nuclei of unequal size.
24. Octonucleate cyst.
25. Quadrinucleate cyst—chromatoids becoming absorbed.
26. Ripe quadrinucleate cyst.

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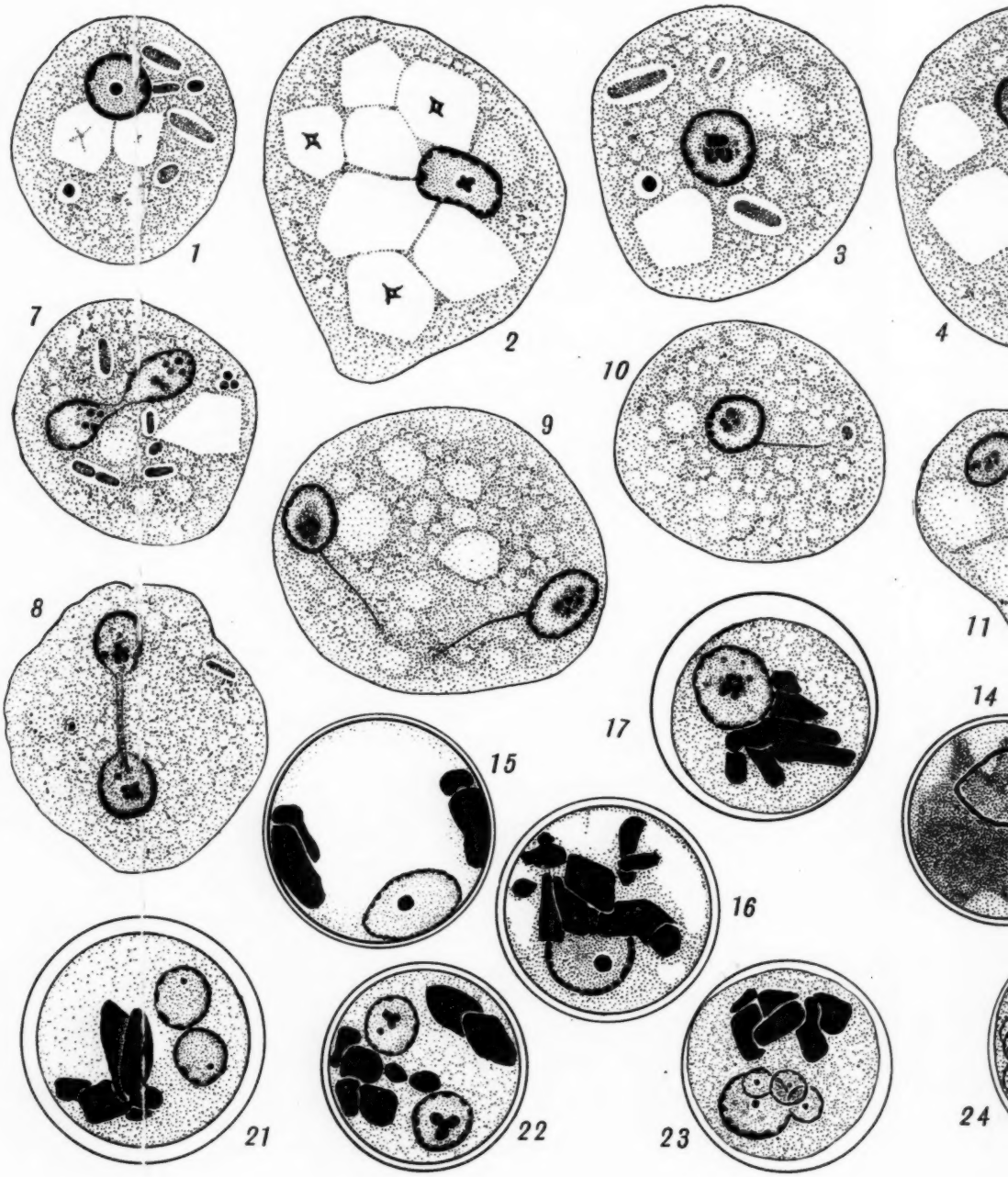
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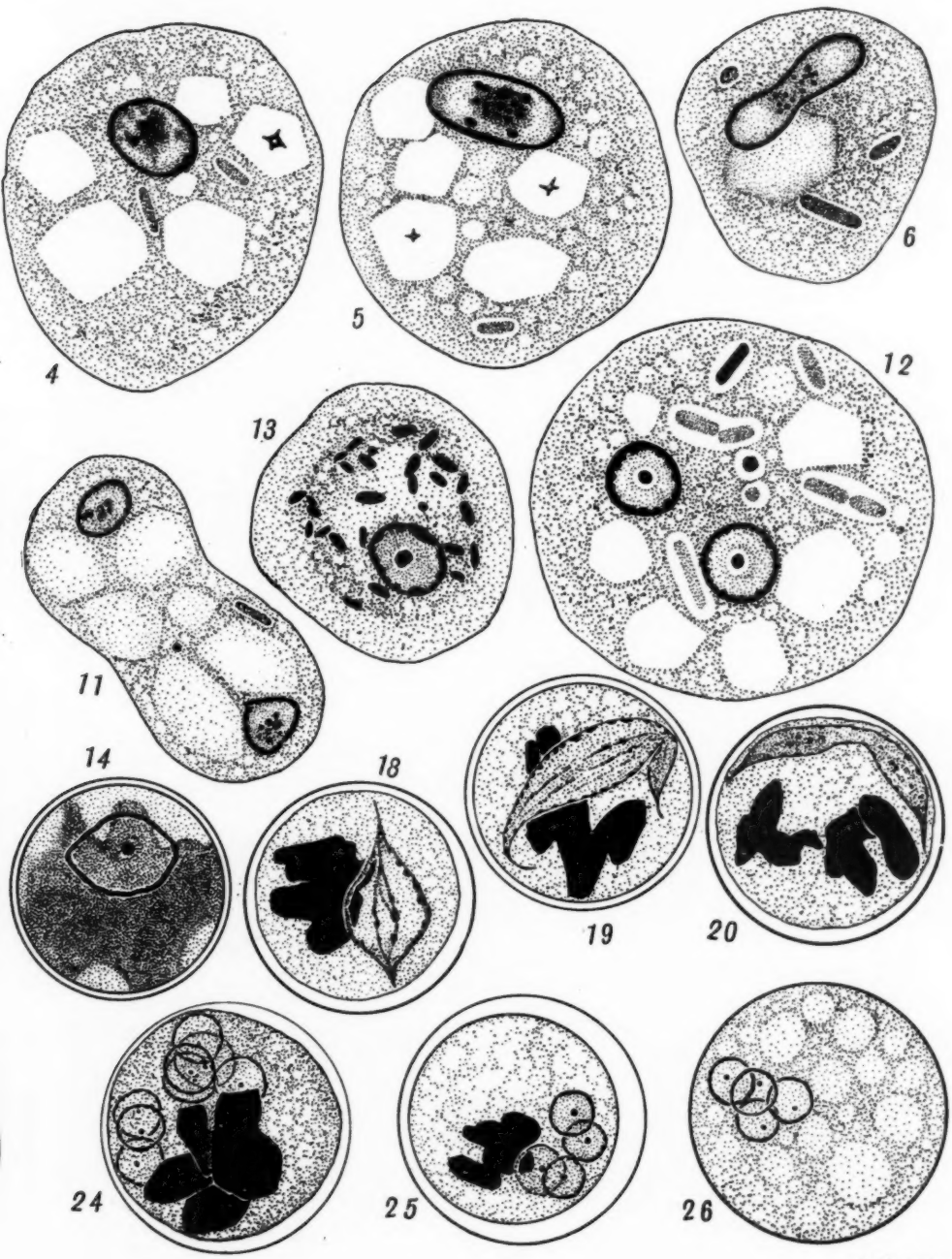
PLATE XI



A. B. DEL.

A NOTE UPON *RETORTAMONAS ROTUNDA* N.SP., AN INTESTINAL FLAGELLATE IN *BUFO VULGARIS*.

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(With 1 Figure in the Text.)

Whilst studying the intestinal fauna of *Bufo vulgaris* I found a small flagellate which hitherto has not been described from this host. It was present in small numbers in the gut of toads from two different localities. The flagellate's body is either almost spherical, or somewhat egg-shaped. It measures 6–8μ × 5–6μ. The nucleus, which lies at the anterior end, is spherical and surrounded by a delicate membrane. Between this nuclear membrane and the central spherical karyosome is a zone which does not stain, but through which fine rays pass from the karyosome to the nuclear membrane. The karyosome stains intensely and homogeneously with iron haematoxylin. Four flagella arise from the anterior end; three being of equal length, about thrice the body length, but the fourth is long, and trailing, and twice the length of the others. They arise in two pairs a distance apart, each pair being inserted into a very small basal granule. The two basal granules are joined by a siderophilic thread. From one basal granule arises a deeply staining axostyle which differs from that of *Trichomonas* and *Eutrichomastix* in being a homogeneously staining rod. It runs throughout the length of the body and sometimes projects slightly from the posterior end. There is no mouth.

A comparison of this flagellate (Fig. 1 A) with *Retortamonas* (*Monocercomonas*) *orthopterorum* (Fig. 1 B) from the cockroach shows great similarity between the two flagellates. I have frequently found *R. orthopterorum* in preparations made from the gut of *Blatta orientalis* (cockroach) and cultivated it for five months in a medium consisting of an inspissated horse-serum slope covered with inactivated horse-serum diluted 1 : 10 in 0·8 NaCl solution. To this medium a little solid sterile rice-starch was added. Although *R. orthopterorum* has no mouth, it ingests starch granules, which adhere to the body surface at the posterior end and are gradually ingested. The cultures produced rich growths of *R. orthopterorum* and needed subcultivating only every 14 days. No cysts appeared. Bělař (1916) has described division in this species, and Grassé (1926) has described small, almost spherical, cysts.

I have been unable to cultivate the flagellate from the toad, as *Tricho-*

monas batrachorum was invariably present in great numbers and multiplied so rapidly that the small flagellate was overgrown.

The toad flagellate is more globular than *R. orthopterorum* (compare Fig. 1 A and B). The flagellates are about of equal size and their nuclei are similar in structure, but that of *R. orthopterorum* is smaller. The flagella are similar in arrangement and comparative length, except that in a few specimens of *R. orthopterorum* three flagella may arise together and the remaining one alone, an arrangement not seen in the toad flagellate. The basal granules in this flagellate are simple, whereas in *R. orthopterorum* they may be bilobed or

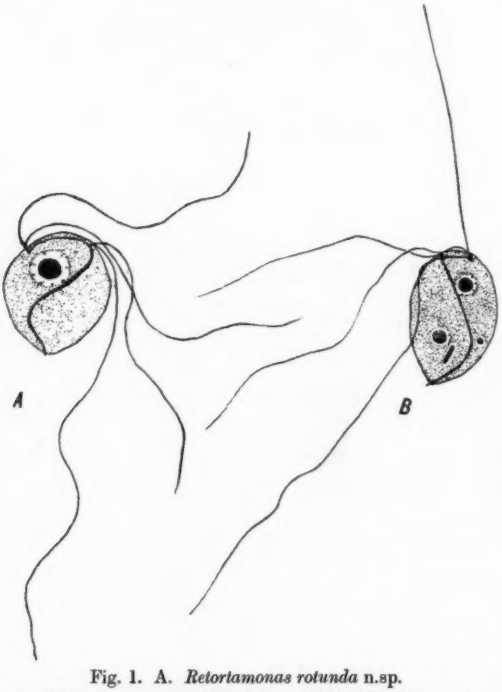

Fig. 1. A. *Retortamonas rotunda* n.sp.
B. *Monocercomonas orthopterorum* (Parisi) Bělař emend.

double. Both flagellates have no mouth and they have the same type of axostyle. They are so similar that they obviously belong to one genus.

The cockroach flagellate was formerly placed in the genus *Monocercomonas* (Bělař, 1916), but Wenyon (1926), who has dealt with the nomenclature of this genus, refers it to the genus *Retortamonas* (Grassi, 1879). In reading Grassi's papers (1879, 1882 and 1883) dealing with this subject, it is difficult to decide exactly which flagellates were included in the various genera mentioned. In the first paper (Grassi, 1879) *Monocercomonas* was probably a synonym of *Trichomonas* and contained only flagellates of this genus; the flagellates of

insects were placed in the genera *Retortamonas* (*R. gryllotalpae*) and *Schedoacercomonas* (*S. gryllotalpae* and *S. melolonthae*)—it is impossible, from his description, to arrive at any definite idea of the detailed structure and exact relationship of these flagellates. In 1882 Grassi gives *S. gryllotalpae* and *S. melolonthae* as one species, *Monocercomonas insectorum*, but his genus *Monocercomonas* 1879 was a synonym of *Trichomonas* and therefore could not be applied to flagellates of different structure. Lastly, Grassi (1883) figures *Monocercomonas insectorum*, and from its appearance it seems to belong to the group of flagellates including the species from the cockroach described by Bělař under the name of *Monocercomonas orthopterorum* (see above), and for which group Wenyon (1926) proposes the amended name of *Retortamonas*. Grassi (1879) gave no illustrations of *Retortamonas* and *Schedoacercomonas*. It seems possible that these flagellates belonged to the same genus, in which case the name *Retortamonas* takes precedence as Wenyon points out; and I concur with him in this. I therefore adopt the generic name of *Retortamonas* for the flagellate from the toad and name it *R. rotunda* n.sp.

A number of species of *Retortamonas* have been described, but, excepting *Monocercomonas caviae* (= *R. caviae*) described by Cunha and Muniz (1921) and mentioned by Pinto (1925) and later renamed *M. hassalli* (Cunha and Muniz, 1927), as the earlier name was shown to be preoccupied, all have been parasites of insects. *R. hassalli* was found in *Cavia porcellus* (Brazilian cavy).

In addition to the species described by Grassi, there are the following forms from insects: Jollos (1911) described *Monocercomonas cetoniae* from the gut of *Cetonia* larvae. Hamburger (1912) described a *Monocercomonas* from the intestine of *Tipula* larvae, this being similar in structure to *M. melolonthae* Grassi 1882 and *M. cetoniae* Jollos. França (1913) described a *Monocercomonas* from the larvae of *Oryctes grypus*, *O. nasicornis* and *Phyllognatus silenus*. Bělař (1916) described in detail the morphology and method of division of *Monocercomonas orthopterorum*. Grassé (1926) studied both *Monocercomonas cetoniae* and *M. melolonthae* and decided that they are identical. In view of Wenyon's revision, the generic name of these flagellates should be *Retortamonas*.

Bělař (1916) believed that the flagellate described by Parisi (1910) under the name of *Trichomonas* (*Trichomastix*) *orthopterorum* was identical with *Retortamonas* (*Monocercomonas*) *orthopterorum*. But as in Parisi's flagellate the axostyle, if present, was similar to that of *Trichomonas* and *Eutrichomastix* its identity with *R. orthopterorum* seems to me to be doubtful.

Chatterjee (1917) described a *Monocercomonas* from dysenteric stools; but the four equally long flagella and the absence of an axostyle makes it improbable that this flagellate belongs to *Retortamonas* (= *Monocercomonas*).

The above-mentioned species from insects, the Brazilian cavy and *Bufo vulgaris* are all similar morphologically, and obviously belong to one genus.

Difficulty arises concerning the nomenclature of the toad flagellates. Dobell (1909) described as *Monocercomonas bufonis* n.sp. a long thin flagellate measuring

10–20μ × 2–7μ, of which he says "The four flagella are equal in length and are all directed anteriorly: that is to say, there is no 'Schleppgeissel' as in *Trichomastix*." This flagellate has no mouth and no axostyle. Dobell says that he "referred the animal to the genus *Monocercomonas* Grassi" because he believed that "the genus *Tetramitus*, to which similar organisms belong, ought to be reserved for free-living forms." *M. bufonis* has since been described by Alexeieff (1911) under the same name, but in 1916 without giving any reason, he created the generic name *Tetramastix* for its reception, removing it from the genus *Monocercomonas*. Chalmers and Pekkola (1918) described a parasite from *Testudo calcarata* under the name of *Protetramitus testudinis* and believed that *M. bufonis* Dobell (1909) belongs to the same genus. These authors therefore transfer *M. bufonis* Dobell 1909 to *Protetramitus bufonis* (Dobell 1909) Chalmers and Pekkola 1918.

The flagellate described by Swezy (1916) as *Polymastix bufonis* had a striated cuticle and for this reason she placed it in the genus *Polymastix*, stating clearly that this flagellate, identified by her with *M. bufonis* Dobell, does not belong to *Monocercomonas* as it has no axostyle, has a striated cuticle and "in most cases, the extranuclear chromidial bodies or parabasal bodies." Tanabe (1925) provisionally accepts Swezy's name for this flagellate.

Bütschli (1883–1887) established the genus *Polymastix* for a flagellate from the gut of the *Melolontha vulgaris* larva, including in his generic characters the presence of a striated cuticle. Hamburger (1912), França (1913), Mackinnon (1912) and Grassé (1926) described *Polymastix* from insects. Apart from the striated cuticle they resemble, in nuclear structure and general morphology, members of the genus *Retortamonas* (= *Monocercomonas*) much more closely than *Polymastix bufonis* (Dobell 1909) Swezy 1916. Grassé decided, however, that there was sufficient difference in structure between the insect flagellates ascribed to *Polymastix* and *Monocercomonas* (= *Retortamonas*) to justify preserving these two genera, but he does not agree that *Monocercomonas bufonis* Dobell 1909 belongs to *Polymastix* as Swezy thought. Whereas Swezy described the presence of cuticular striations in the toad flagellate, Tanabe (1925) could demonstrate them in material stained by Wright's method only. In all other details he found the flagellate similar to that described by Dobell. I have seen very heavy infections of Dobell's flagellate in individual toads, but have never observed cuticular striations, though hitherto I have not used Wright's stain. Grassé is convinced that *Monocercomonas bufonis* Dobell 1909 cannot be included in *Polymastix* owing to the great development of the parabasal apparatus; he therefore places it in a separate genus and accepts Alexeieff's (1916) generic name of *Tetramastix*. In this genus he places *T. bufonis* (Dobell 1909), and creates a special species, *T. swezyi*, for the flagellate described by Swezy, which, he points out, differs from *T. bufonis* in the striated cuticle and the arrangement of the blepharoplasts. He also includes *Protetramitus testudinus* Chalmers and Pekkola 1918 as a species (*T. testudinis*) of this genus.

SUMMARY.

In *Bufo vulgaris* there is a small flagellate similar to species described from insects and the Brazilian cavy. Such flagellates were formerly placed in the genus *Monocercomonas* but the generic name has been amended to *Retortamonas* Grassi 1879 by Wenyon (1926). The toad flagellate which I name *R. rotunda* n.sp. differs in structure from *Monocercomonas bufonis* Dobell 1909, which Grassé (1926) has removed to the genus *Tetramastix* Alexeieff 1916.

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A CESTODE, *OOCHORISTICA KHALILI* N.SP. FROM A SNAKE, *PSAMMOPHIS SCHOKARI* FORSKÅL.

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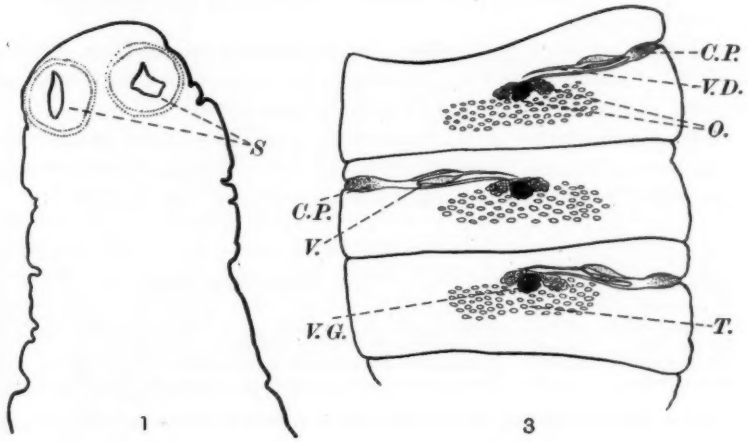
(With 3 Figures in the Text.)

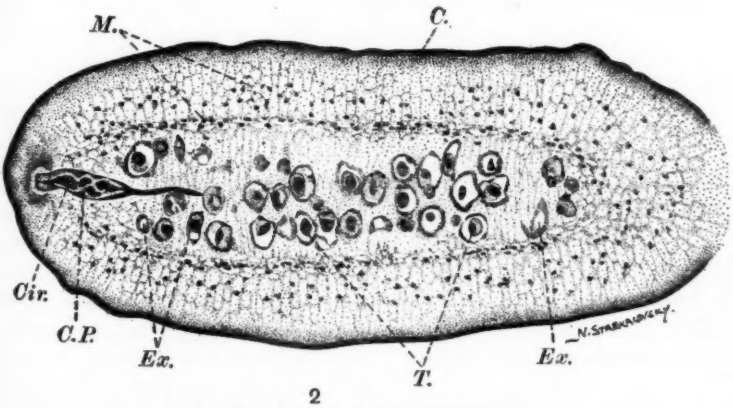
The cestode herein described was obtained from the intestine of a Schokari Sand Snake which died on 16. xii. 1929 at the Zoological Gardens, Giza, after being 6 months in captivity. The material (Museum No. 1717) was placed at my disposal by Prof. Khalil for study in the Department of Parasitology.

Sections (transverse) were cut to a thickness of 5 or 6 μ and stained with haematoxylin and eosin.[1] Scolex (Fig. 1) like a truncated cone with anterior border slightly convex, its width is 0·33 mm. immediately anterior to the suckers, 0·46 mm. across the sucker's diameter and 0·57 mm. at its broadest portion which is followed by a short slender neck. Suckers, four, unarmed, circular, deeply set and non-pedunculated (Fig. 1), diameter of each 0·20–0·22 mm. Rostellum absent. Neck (Fig. 1) 0·90 mm. long, 0·46–0·80 mm. broad. Cuticle (Fig. 2) only 8 μ thick. Muscles, both circular and longitudinal (Fig. 2), poorly developed. Proglottides (Fig. 3) collar-shaped, broader than long, their posterior corners overlapping next segment, average breadth 1·93 mm. Excretory vessels (Fig. 2), unbranched, run zigzag on each side of chain. Nerve trunks lateral to excretory vessels curving within proglottis with convexity toward margin. Genital pores (Fig. 3) lateral, irregularly alternating, lie at junction of anterior with posterior two-thirds of proglottis margin. Cirrus pouch spindle-shaped (Figs. 2 and 3), 0·286–0·318 mm., reaches inward to limit of excretory vessels. Cirrus (Fig. 2) 0·120 mm. long. Vas deferens (Fig. 3) a thin slightly wavy tube. Testes (Figs. 2 and 3) 44–61 in number (average 52 per segment), lie behind and on either side of female sexual glands, they are ellipsoid with long axis transverse and are 0·063–0·079 mm. long by 0·030 mm. broad. Vagina (Fig. 3) a thin tube opening into cirrus pouch at its posterior border, runs parallel and posterior to it and vas deferens to ovary. Shell gland and receptaculum seminis not found. Ovary (Fig. 3) compact, bipartite and central in position, each part irregularly oval. Vitelline gland (Fig. 3) lobulated, fairly compact, situate behind and between the two parts of ovary, with long axis directed more or less antero-posteriorly. Uterus, in ripe segments, seems to have been dissolved leaving ovi-capsules lying in the parenchyma. Ovi-

[1] No complete strobilus was available for examination.

capsules consist of three envelopes enclosing one ovum, circular, measuring 0·070–0·080 mm. Embryo, circular in outline, measures 0·040–0·048 mm., possesses three pairs of hooklets. Sexual canals pass between excretory vessels.





Figs. 1–3. *Oochoristica khalili*, n.sp.

Fig. 1. Scolex, × 53. *S.* Suckers.

Fig. 2. Transverse section of a segment, × 60. *C.* cuticle; *Cir.* cirrus; *C.P.* cirrus pouch; *Ex.* excretory vessels; *M.* musculature; *T.* testes.

Fig. 3. Three consecutive segments, × 27. *C.P.* cirrus pouch; *O.* ovary; *V.* vagina; *V.D.* vas deferens; *V.G.* vitelline gland.

From the above description and on account of the characters of the scolex, the genital pores, the uterus and ovi-capsules and the presence of one set of sexual glands and their median position the cestode described is referable to the genus *Oochoristica* Lühe 1898 of the sub-family Linstowinae Fuhrmann

1907 of the Anoplocephalidae. It differs, however, from all known species of the genus in one or more characters. Thus: *Oochoristica amphisbaenae, ichneumontis, lemuris, megastoma, rostellata, surinamensis, tetragonocephala* and *zonuri* all have not less than 60 testes each and in some, *e.g. O. megastoma*, there are 160–200 testes.

On the other hand, *Oochoristica agamae, amphisbetata, bivittata, crassiceps, didelphidis, incisa, theileri* and *tuberculata* have much less than 60 or even 50 testes each and in some, *e.g. O. bivittata*, they number but 5–8.

With the exception of *O. herpestes* the remaining known species of the genus, though they may resemble the cestode herein described in the number of testes, differ from it in other important characters set out in Table I:

Table I.

Oochoristica sp.	No. of testes	Differential characters
(1) *amievae*	ca. 50	Length 25 mm.
(2) *brasiliensis*	40–50	Cirrus pouch 0·15 mm. long
(3) *bresslaui*	ca. 60	Excretory system highly branched
(4) *erinacei*	30–50	Horseshoe ovary and crescentic vitelline gland
(5) *trachysauri*	ca. 60	Testes in two distinct groups on either side of vitelline gland

When the cestode (*O. khalili*) herein described is compared with the only remaining species of the genus, viz. *O. herpestes*, it is clear, by reference to Table II, that it represents a new species.

Table II.

	O. herpestes	*O. khalili*
Host	*Herpestes sanguineus* Rupp. Mammalia; Carnivora	*Psammophis schokari* Forskål Reptilia; Ophidia
Neck	Absent	Present
Suckers	Heart-shaped	Circular
Excretory vessels	Slightly branched	Not branched
Convexity of nerve in proglottis	Away from proglott. margin	Towards proglott. margin
Alternation of genital pores	Regular (Kofend, 1921) Irregular (Baer, 1927)	Irregular
Cirrus pouch	0·210–0·220 mm.	0·286–0·318 mm.
No. of testes	50–60	44–61
Length, direction of long axis of testis	0·120 mm. dorso-ventral	0·063–0·079 mm. transverse
Vas deferens	Strongly convoluted	Slightly wavy
Relation of sex canals to excr. vessels	Dorsal	Between them

In conclusion, I regard the cestode herein described as a new species to which I give the name of *Oochoristica khalili* in grateful recognition of the valuable help and guidance accorded me by Prof. Khalil.

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ON THE TECHNIQUE OF CESTODE STUDY.

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(With 2 Figures in the Text.)

The accurate identification of cestodarian and cestodan helminths depends so much upon the correct interpretation of their internal morphological features that a rapid yet satisfactory routine technique in their preparation for microscopical examination is much to be desired. Not all workers publish their technical methods, and, among those who do, no two seem to be in agreement. Some of the collection and preservation methods, in particular, which are advocated, whilst admirable for museum specimens or histological study, are impracticable under the conditions of a field survey, where many host animals have to be quickly examined and rapidly accumulating specimens are preserved and studied by methods which will not strain unduly the limited facilities of a field laboratory or a small biological station.

The following notes, based upon the author's experience under such conditions in western Canada, may be of interest to other workers in parasitology.

Collection and fixation.

Methods of collecting cestodes cannot vary greatly whatever the host type happens to be, and probably all collectors use some form of decantation procedure. The alimentary tract of the host is slit open, freed from the large, obvious helminths, and is then cut into pieces which are soaked in water or in sodium chloride solution. Finally the supernatant liquid is poured off and the sediment is examined over a black surface, any visible helminths being picked out with a brush or a hooked needle. Variants of this procedure have been described by Meggitt (1924), Hunter (1927), Essex (1927), Southwell (1930) and others.

Meggitt advises that the host gut be cut into 4 in. pieces, each of which should be shaken in fresh water heated to 40° C.; he deprecates the use of salt solution or of cold fresh water. Southwell splits the whole intestine and washes it in running fresh water for 1 or 2 hours, the débris being again washed for a further 2 hours, and the cestodes picked out; he opposes the use of salt solution, but favours the use of cold fresh water. Hunter divides the host alimentary tract into sections labelled "stomach," "upper third," and so forth, places each section in a bottle of normal saline and shakes it; the supernatant saline is then decanted and the débris examined; or he empties the contents of

stomach and intestine into a conical net of bolting cloth and washes thoroughly under a tap stream, the contained helminths thus becoming concentrated in the net. Essex removes the larger parasites and débris from the tract, scrapes the gut contents into a quart jar of water, shakes thoroughly, strains the material through a plankton net, runs a stream of water through it for some time, then examines the sediment.

The chief objection to most of the decantation methods advocated is the relatively long period of time during which the material is exposed to the influence of an abnormal medium. An intestinal helminth lives in a medium whose osmotic pressure fluctuates around eight atmospheres, and the internal osmostic pressure of the helminth is in equilibrium with this. In solutions of low osmotic pressure, such as fresh water or salines of lower than 0·001 molar concentration, the worm expands, becomes flaccid, owing to endosmotic passage of water into the worm, and commences to disintegrate, such disintegration progressing from the abothrial extremity forwards and from the cuticle inwards. In solutions of $CaCl_2$ or NaCl of molar concentrations between 0·01 and 0·2, or in Ringer-Locke solution, the activity period prior to disintegration is longer than in fresh water; owing possibly to the existence of a Donnan equilibrium between the internal Cl' content and that of the surrounding medium, the worm can to some extent adjust its internal osmotic pressure towards equilibrium with the medium, and may live actively expanding and contracting for several hours or even days. Eventually of course a gradual muscular contraction commences which is followed by disintegration, or the worm may pass comparatively quickly from activity to endosmotic immobility and disintegration. If, therefore, it is necessary to postpone fixation of helminth material for some hours after removal from the host, the most suitable medium is decimolar NaCl, rather than fresh water, to which has been added 5 per cent. of egg white. There is no evidence that the egg white serves as a food, but the presence of a colloidal substance in the saline appears to retard the antagonistic action of the kations of neutral salts upon cestode activity.

Routine preferred by the author.

(*a*) *For mammals and birds.*

The routine preferred by me in obtaining material from mammals and birds is as follows. The alimentary tract of the host is exposed, ligatured at the junction of stomach (or gizzard) and duodenum, and of small intestine and large intestine, and the small intestine removed. This is now straightened out, by snipping the mesenteries, upon a black surfaced board provided with a scale in inches or millimetres. Commencing at the pyloric end of the intestine, a length of 12 in. is slit open with blunt-nosed scissors and any obvious helminths removed to decimolar NaCl, their positions in the intestine relative to the pylorus being noted by comparison with the scale. The length of intestine is then detached from the rest and its contents squeegeed with a camel's hair mop

into decimolar-NaCl. The rest of the intestine is dealt with similarly. The helminths obtained are removed almost immediately from the saline, preferably by an assistant, stretched upon a glass plate and fixed in the manner described below. Cestodes which adhere to the host mucosa can frequently be induced to relax their hold by flooding them with saline. If this is ineffective, they should be fixed *in situ* or should be removed, together with the area of mucosa to which they adhere, to a glass plate.

During an examination of large numbers of *Lepus americanus*, by a colleague, this routine was expedited by passing the intestine when taken from the host over a ground-glass plate illuminated from below. Such large helminths as *Cittotaenia* were readily located and removed through a slit. The intestine, or such portions as had not been slit, was then connected to the tap stream and its contents flushed out into a large beaker, washed for 2 min., decanted, and examined. Nematode worms are readily removed by this method, and a comparison of the number of individuals of the strongyloid worm *Nematodirus* per host disclosed by this method, with the number found by microscopical search of the mucosa, showed a difference of only 5 per cent. in favour of the laborious microscopical method.

(*b*) *For fishes.*

For the rapid routine examination of the alimentary tracts of teleostean fishes, decantation methods are unsatisfactory, since the cestode parasites of such fishes live commonly within the pyloric caeca and are not readily removed except by the tedious method of slitting open each caecum and removing the helminth with a needle. A more satisfactory procedure is to place the alimentary tract upon a black surfaced table, to snip off the tips of the caeca and to slit open the gut. Each caecum can now be washed out by a fine but powerful stream of fresh water issuing through a finely nozzled cannula inserted in the internal opening of each caecum in turn. The helminths are readily dislodged and even the smallest are easily discernible against the black surface. The whole operation takes only 2 or 3 min. Intra-caecal dibothriocephaloids do not usually adhere so firmly to the caecal mucosa as to resist expulsion by a water jet. In the rare cases where they do resist, as in the species of *Eubothrium*, which lives in *Lota lota* with its *scolex deformatus* imbedded in the host mucosa, no method other than tedious needle dissection is of any avail.

The tetrarhynchoid and phyllobothrioid worms of elasmobranch fishes, adherent to the spiral valve of the intestine, should be fixed *in situ*, whereupon they usually relax their hold.

During an examination of large numbers of fishes for the plerocercoid stages of *Diphyllobothrium latum* and *Triaenophorus tricuspidatus* which occur in the epaxonic and hypaxonic musculature of the host, the following method was found sufficiently rapid and accurate to permit the examination of several hundred fishes per day. Each fish is held by the head; a horizontal cut with a filleting knife removes the dorsal fins and the underlying muscle strip; three

vertical incisions on either side, running from dorsum to ventrum, separate the skin from the underlying musculature and divide the musculature into three sheets on each side. Any plerocercoids in the muscle sheets can readily be seen, even though the muscle sheets are not removed from the fish. The whole operation takes about 1 min., but frozen fishes may require preliminary thawing.

Fixation.

The helminth material obtained by the various collection methods described above should be fixed as soon after removal from the host as possible. The fixing solution should be efficient, cheap, readily procurable, portable in any concentration, and should not require post-fixation removal. Fixatives which have to be prepared fresh for each batch of material, which require ingredients not readily procurable, which require post-fixation removal, are unsuitable for field laboratory routine.

Of the four common types of fixative used for tissue fixation—formalin, sublimate, picric and chromic fixatives—the one which comes nearest to satisfying the requirements laid down above is neutralised formalin, diluted with decimolar sodium chloride. It is not a good precursor of haematoxylin stains, and, unless neutralised, it will dissolve cuticular spines and calcareous bodies, but it is cheap, easily obtainable, portable even in concentrated form, and does not require immediate post-fixation removal. Cestode material can be satisfactorily fixed by immersion for 1 hour in 10 per cent. solution, and preserved indefinitely in 5 per cent. solution. Better results are obtainable by immersing the material in 10 per cent. for 12 hours, then in 5 per cent. for 24 hours, then washing for 2 hours in running fresh water and gradually transferring through 30, 50, 70 to 80 per cent. alcohol for permanent storage.

Sublimate fixatives such as acetic acid-corrosive sublimate, although excellent for material which is to be studied histologically, are not good precursors of the safranin staining technique described below, and—great disadvantage under field conditions—are alcohol consumers, since the fixed material requires much post-fixation soaking in alcoholic iodine. Picric fixatives, such as Bouin's solution, are also alcohol consumers. Chromic fixatives, such as Zenker's solution, although probably the best fixatives for cestode material, require much post-fixation washing of the material in running water in darkness and so are usually impracticable for field laboratory procedure.

After a trial of various methods of applying the fixative—shaking the worm in fixative, suspending it in fixative (Baylis, 1922), manipulating it in fixative with rubber-gloved fingers (Gough, 1911), fixing between sheets of glass (Southwell, 1930)—the most satisfactory method in my experience is that of *painting* each helminth with a brush soaked in hot water (60° C.) and then transferring it to cold 10 per cent. solution of saline-formalin.

A tetrarhynchoid, phyllobothrioid or taenioid cestode, if adherent to the host mucosa, may be fixed *in situ*; a dibothriocephaloid or proteocephaloid type is better stretched upon a glass plate and fixed there; in either case the

worm is held by pressure on the scolex with a brush soaked in hot water, whilst the strobila is stroked slowly from scolex to abothrial extremity with another charged brush; the worm is then transferred to cold fixative, left for at least an hour, and then transferred to the final preservative solution, or, after the hot-water treatment it may be taken straight into the staining solution.

Minute forms may be dropped into hot water and left for a few moments, then removed with a pipette into cold fixative.

The comparative effect of painting as compared with flooding (with hot

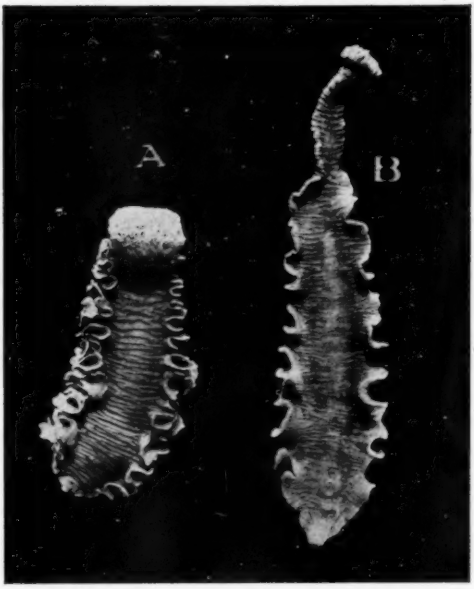

Fig. 1. Twin specimens of *Gyrocotyle urna* fixed by (A) flooding with hot 10 per cent. formalin from a pipette, (B) painting with hot 10 per cent. formalin.

10 per cent. formalin) is illustrated in Fig. 1, upon *Gyrocotyle urna*, a difficult helminth to fix in an expanded condition. Both specimens were of equal length when alive, and were fixed *in situ* attached to the intestinal mucosa of *Hydrolagus colliei*, a Pacific chimaeroid fish.

Staining.

There appears to be some variance of opinion as to the respective merits of carmine and haematoxylin stains for the *in toto* staining of cestode material. Many workers, such as Millzner (1926), Gough (1911), Meggitt (1924), Southwell (1930) for example, claim excellent results with such stains as acetocarmine, alum carmine, borax carmine, alum cochineal. Other workers find such stains

to lack penetration power and prefer haematoxylin stains of the Delafield or Ehrlich formulae.

In my experience, a long trial of various carmine stains with dibothriocephaloid and proteocephaloid material failed to give satisfactory results. On the other hand, excellent results were obtained with alum cochineal upon formalin-fixed taenioid material which had previously been well washed in running water and which was stained progressively with a solution of two drops of stain to 10 c.c. of water. Alum cochineal, however, is unsuccessful with Bouin-fixed material and not altogether satisfactory with sublimate-fixed material.

The extent to which carmine stains are successful with cestode material would appear to depend to some extent upon the compactness of the internal structures. The compact vitellarium and germarium of the taenioid, for example, seems to absorb the carmine type of stain to a much greater extent than do the diffuse vitellaria and loosely lobed germarium of the dibothriocephaloid.

Two methods of staining cestode material may be made use of. In the case of cestodes which are relatively thick and fleshy, such for example as *Eubothrium*, *Diplocotyle*, *Cyathocephalus*, *Triaenophorus*, or in the case of cestodarian helminths, the staining solution should be a concentrated one and the material should be deliberately overstained by immersion for several days. Differentiation should be carried out rapidly in 10 per cent. alcoholic HCl or $H_2C_2O_4$, used hot if necessary. On the other hand, if the cestode is thin and ribbon-like, as in most species of *Diphyllobothrium* and *Proteocephalus*, a very dilute staining solution can be used. The material is immersed for 12–18 hours and when removed from the solution may appear insufficiently stained, to judge from the superficial appearance. Subsequent dehydration and clearing, however, will usually develop a sharply defined picture of the internal genitalia. Differentiation is usually not required.

A compromise between the two methods is provided by the procedure recommended by Hoff and Hoff (1929) for unusually thick forms. Such material is pinked with borax carmine, dehydrated, imbedded in paraffin wax, and the cuticle and underlying muscle layer of each surface is sliced off with a microtome. The material is then removed from the wax by soaking in xylol, brought into distilled water, and stained in the way suggested for thin cestodes. The method is tedious and not to be recommended except as a last resort.

When differentiation in acidulated alcohol is rapidly carried out and the immersion period in the acid solution is a matter of seconds, subsequent passage through alkaline alcohol is unnecessary; in the case of haematoxylin-stained material, such alkaline treatment tends to darken the preparation, and if ammonia be used as the neutraliser the material may turn quite black and require bleaching in oxalic acid and restaining.

After differentiation, the material should be washed in running water for an hour, soaked for an hour in 50 per cent. alcohol, for another hour in 95 per

cent. alcohol, and then transferred to beechwood creosote. The author finds beechwood creosote preferable to xylol, methyl salicylate, anilin oil, or other recommended clearing agents, for such material. It clears rapidly, is of high refractive index, and does not require that the material be completely dehydrated. The material should soak in creosote for a period varying from 30 min. to several weeks, the longer the better, and then should be mounted in a creosote solution of Canada balsam.

Brittle, cork-stained material can be softened and decolorised to some extent by soaking in 10 per cent. citric acid, in 5 per cent. sodium hydroxide, in glycerine, or, according to Meggitt (1924), in beechwood creosote (5 days) followed by 10 per cent. alcoholic HCl (5 days). Such softened material, though amenable to *in toto* staining, is unsuitable for microtome sectioning, since it rapidly hardens again when dehydrated and becomes too brittle to section.

Material which has been stained in haematoxylin and cleared in creosote, is excellent for microtome sectioning, although, if the period in creosote has been prolonged, the sections may be somewhat brittle and adhere with difficulty to the slide. The material is prepared by soaking for 1 hour in xylol, for 1 hour in a 50 : 50 mixture of xylol and parawax, for 1 hour in melted parawax (M.P. 45° C.), for a further hour in fresh parawax, and is then imbedded in wax of M.P. 52° C. in a paper box surrounded by crushed ice. The sections usually require no further staining.

Sections which have been prepared from unstained or from superficially stained material, may be stained by any of the four following stain combinations: Delafield haematoxylin and eosin, Delafield haematoxylin and safranin and picric acid, acid fuchsin and picro-indigo-carmine, eosin and picro-indigo-carmine.

The first method is a routine procedure in most biological laboratories and need not be described here.

In the second method, the sections, after wax removal, are brought gradually into distilled water and stained by immersion for 12–18 hours in Delafield haematoxylin diluted with an equal bulk of distilled water. The stained sections are rinsed in running water and transferred to a solution of safranin, 2 grm. in 60 c.c. of 50 per cent. alcohol. The material adsorbs the safranin almost instantaneously so that the sections need not remain in the solution for longer than 1 min., although it may be noted that sublimate-fixed or Zenker-fixed material does not adsorb safranin so readily as does formalin-fixed or Bouin-fixed material. The sections are now rinsed in 50 per cent. alcohol, passed through 70 and 95 per cent. to absolute alcohol and from thence transferred to a solution of picric acid in absolute alcohol. Five drops of a solution of 1 grm. of picric acid in 15 c.c. absolute alcohol being diluted with 5 c.c. of absolute alcohol. After an immersion of 1 min. duration the sections are rinsed in absolute alcohol, cleared in xylol and mounted in xylol-balsam.

In the third method, the sections, after wax removal, are brought into distilled water and stained with aqueous acid-fuchsin (1 per cent.) for 20–30 min., rinsed in distilled water, and transferred to picro-indigo-carmine (*i.e.* 5 grm. of indigo-carmine in 150 c.c. of saturated aqueous picric acid) for 5–10 min. until a green tinge begins to appear against the red background of the acid fuchsin. The sections are now rinsed *quickly* in distilled water, immersed in 70 per cent. alcohol until the surplus stain ceases to come out, transferred to absolute alcohol, cleared in carbo-xylol, and mounted in xylol balsam.

In the fourth method, the procedure is similar to that of the acid-fuchsin method, but a 2 per cent. solution of eosin in distilled water is used instead of the acid-fuchsin solution, the period of immersion being 24 hours.

Reconstruction.

An understanding of the number, size, shape of such structures as testes, vitellaria, the shape and size of germarium and uterus, the interrelations of genital ducts, the distribution of the nervous and excretory systems, and so forth, can often be obtained only by the study of a magnified copy of the structure in question, obtained by tri-dimensional reconstruction from microtome sections.

Such a reconstruction may be plastic, that is to say it may be an actual model prepared from some material that can be cut or moulded—wood, wax, clay, paper, celluloid, gelatine—or it may be graphic, the object being represented by contour lines upon a plane surface or within a transparent cube formed of glass or celluloid plates. A general account of various reconstruction methods has been published by Peters (1922) and a detailed account of Born's wax plate method and Kerr's glass plate method has been given by Norman (1923). Since that date, however, the application of reconstruction methods to cestode material has been greatly facilitated by certain improvements in the wax plate method and celluloid method, suggested by Lebedkin (1930), and by the method of using gelatine as a medium, invented by Lapin (1927). These recent contributions to the technique of reconstruction may be alluded to briefly.

The original method of making wax plates suggested by Born in 1883 is still one of the most convenient, although one of the slower methods of preparing them. A rectangular metal dish is used as a mould. Its surface area and the specific gravity of the wax-turpentine mixture being measured, it is easy to calculate the mass of wax required to make a plate of this surface area and of given thickness. The dish is filled with boiling water to the depth of half an inch, and the requisite mass of melted wax is poured upon the water surface, the hot wax being smoothed by passing over it a glass slide. As soon as the wax has solidified, but whilst it is still soft, it is freed from the sides of the dish, removed to a flat surface and allowed to harden.

The model is built up by assembling a consecutive series of waxen copies of

a corresponding series of microtome sections of the object, the thickness of each wax plate representing the product of the section thickness and the magnification. In actual practice, despite the use of so-called orientation lines, there is some difficulty in assembling the plates so as to ensure that the parts of the model are in accurate correlation with one another. The merit of Lebedkin's contribution to reconstruction technique is that it substitutes for the orientation lines a series of loci on each section, and accurate assembly of the plates requires only that the loci on a section lie exactly below or above those of adjacent sections.

A block of paper sheets is fastened to a smooth board by a metal band placed across the block and parallel to one short side, the band being held to the board by four screws passing through the block. The first section is projected upon the uppermost sheet and its outline drawn. The second section is then projected upon this drawing and fitted as accurately as possible to it. The uppermost sheet is now turned forward and the outline of the second section is drawn. The third section is then projected upon and fitted to this drawing. The second sheet is then turned forward and the outline of the third section is drawn. That is to say, each section image is fitted to the outline of the previous section image before it is itself outlined. If a section be damaged or missing, its outline can usually be reconstructed by combining the outlines of the adjacent sections. As each sheet is turned forwards, the plane of the drawing surface is removed from the plane of the projecting lens by the thickness of one paper sheet; at the turning of every fifth sheet, therefore, it is advisable to place five loose sheets between the drawing board and the table.

When all the sections have been drawn, three points are selected upon the uppermost drawing and through each of these points a needle is driven vertically through the block so as to puncture each underlying sheet; to ensure that the punctures thus made lie in an exact vertical plane, a needle guide may be constructed from a copper tube, a little wider than the needle and half its length, kept vertical by attachment to a basal plate.

The positions of the punctures on each drawing are now marked by crosses. When, as eventually happens, the punctures appear on a drawing *outside* its outline, another series of three points is selected on that drawing and another series of punctures made in the underlying drawings, the positions of these second punctures being indicated by crosses of a different colour or a different shape. Sometimes a third series of punctures may be required. In any case, if the assembled object is likely to exceed in thickness the length of the pins which will be used in fastening the plates together, it is advisable to select fresh points on, say, every twelfth drawing.

Each drawing with its crosses is now transferred to a wax plate by means of carbon paper; the position of each cross on a wax plate is now marked by a needle puncture and the surplus wax of each plate is cut away. The plates are now assembled one on top of another and are connected by fine entomological pins, 3–3·5 cm. long. Commencing with the basal plate, a pin is driven

through each puncture. The next plate is fitted upon the projecting pins so as to fit exactly upon the basal plate. Plate after plate is thus rapidly placed in position (Fig. 2). When fresh pins have to be inserted, this should be done whilst the original pins are still protruding. When the model is complete, any protruding pins can be cut away and the surface of the model can be smoothed over with a hot spatula.

The opacity of such a wax model can be overcome to some extent by a roentgenographic method devised by Politzer (1928). Thus, if it is desired to determine the shape of some internal structure which will be concealed by the opaque wall of the model, the outline of the structure is cut from each plate before the model is assembled. As the model is assembled, the cavity thus left

Fig. 2. Plastic reconstruction of scolex of *Diplocotyle olrikii* in process of assembly by Lebedkin's method.

in it is painted with a mixture of two parts of vaseline and three parts of barium sulphate. In the completed model, therefore, the inner surface of the cavity will have a layer of barium sulphate and the cavity, which of course duplicates the shape of the structure, will show as a black shadow in an X-ray photograph.

Much to be preferred over wax, for reconstruction of a cestode proglottis, in which it is usually necessary to emphasise the internal genitalia, is celluloid or gelatine. Celluloid is used in the form of plates 0·5 or 1·0 mm. in thickness which are assembled by a method similar to Lebedkin's wax plate method as regards section orientation, but in which acetic acid takes the place of pins. The outline of each section of the object is first drawn upon paper and then transferred as a needle incision to the celluloid plate. Orientation points are

selected on each section, as in the wax-plate method, and are marked on the celluloid plates by crosses *on each side* of the plate, the crosses being made with Lyon's blue dissolved in glacial acetic acid. Internal structures are indicated either by cutting out their outlines from each plate, so as to have spaces in the finished model, or by colouring their outlines on the celluloid with pigments dissolved in glacial acetic acid.

The surplus celluloid is cut from each plate with scissors. The last plate to be drawn is placed upon a glass plate illuminated from below and its surface is painted with glacial acetic acid. The succeeding plate is placed upon it, oriented by apposition of the crosses, and squeezed firmly to the acid-painted surface of the lower plate by pressure for a few minutes with a heavy glass plate. The remaining plates are built up similarly one on top of another, each being cemented to its neighbours with acetic acid. It is convenient to assemble the plates in packets of six, and then to assemble the packets. The finished model should be smoothed with a fine file and painted with a thick solution of celluloid.

Gelatine reconstruction, by the method described by Lapin (1927), is cheaper than the celluloid method, and although somewhat slower in procedure, gives excellent results. The method consists essentially of building up the model with plates of gelatine which have been hardened by immersion in formalin, the plates being cemented together with melted gelatine.

Ordinary culinary gelatine is soaked overnight in ten times its weight of water, and is then melted over a water bath, the melting point being 29–30° C. A few drops of 5 per cent. chloral hydrate solution may be added to inhibit fungal growth. To each litre of gelatine there is now added the albumen from two hen's eggs diluted with double its bulk of water. The mixture is thoroughly stirred and then heated for 1 hour on the water bath until the egg albumen has settled. The mixture is then filtered through two thicknesses of wet filter paper. Coloured gelatine may be prepared by the addition of water colours to such clarified gelatine.

The plates are prepared each upon a smooth glass surface, old photographic negatives, when well-cleaned, serving admirably. The glass plate is held within a rectangular metal frame so as to form a loose bottom to a box $\frac{1}{4}$ in. deep. The volume of melted gelatine required to make a plate of the required dimensions and thickness is now calculated, and a number of such quantities are measured from the stock supply and placed each in a test tube kept hot in the water bath. The mould is held horizontally in the hand and the requisite volume of melted gelatine is poured into it, the whole mould being moved about so as to ensure an even thickness of plate. When the gelatine commences to coagulate, the mould is placed in a refrigerator for 2 hours, after which it is immersed in a 10 per cent. solution of formalin for 1 week.

The sections are outlined directly upon the plates with Indian ink, either before or after the immersion in formalin. The surplus gelatine is not however cut away until after the immersion, and should be carried out with the plate

lying under water. The outlines of any internal structures are cut from the plates at the same time, the plates are removed from the water and dried, and the cavities are filled with coloured gelatine dropped from a glass rod, care being taken that the surface of the filling is concave. After removal of surplus gelatine and filling of the cavities, the plates are returned to the formalin bath.

The separation of the gelatine plates from their glass bases is done under water with an old microtome knife. The two plates which will be the innermost plates of the model may however be left still attached to their glass bases, as each half model will be assembled separately. Each half model after assembly is placed for 1 hour in the refrigerator and is then immersed in formalin for 12 hours. It is then removed from the formalin, dipped in melted gelatine, and returned to the formalin bath for 6 hours. Each half model is now removed from the formalin, and detached from its glass base under water. The two halves are now cemented together with melted gelatine, and the finished model is preserved in 10 per cent. formalin in darkness.

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ON A TREMATODE, *ITYOGONIMUS LORUM* (DUJ. 1845), WITH NOTES ON THE OCCURRENCE OF OTHER TREMATODE PARASITES OF *TALPA EUROPAEA* IN THE ABERYSTWYTH AREA.

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(With Plate XII, containing Figs. 1–5.)

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I. Introduction.

Two distinct species have been ascribed to the genus *Ityogonimus*: *I. lorum* (Dujardin 1845) and *I. talpae* (Goeze 1782). The difference between these two species was pointed out by Dujardin in 1845. In the case of *I. talpae* the ventral sucker is about one-third the diameter of the oral sucker, and is situated behind the anterior termination of the yolk glands. In *I. lorum* the ventral sucker is larger than the oral and lies completely in front of the yolk glands. Dujardin named the former *Distoma ocreatum* and the latter *I. lorum*. Witenberg (1926), in further researches on the species, came to the conclusion that the first-named was identical with *Distoma talpae* of Goeze (1782), and as a result of his work there now exist two species of *Ityogonimus* occurring in the mole, namely *I. talpae* and *I. lorum*. The synonyms of *I. talpae* (Goeze 1782) between the dates of 1782 and 1910 are as follows: *Distoma talpae* (Goeze 1782), *Cucullanus talpae* Müller 1787, *C. ocreatus* Shrank 1873, *Monostomum ocreatum* Zeder 1800, *Distoma ocreatum* Duj. 1845, *D. lorum* Melnikow 1865, *Dolichosomum lorum* Looss 1900, *Ityogonimus lorum* Looss 1907, and *I. lorum* Gonder 1910.

The synonyms of *Ityogonimus lorum* (Duj. 1845) are *Distoma lorum* Duj. 1845 and *Ityogonimus filum* Looss 1907.

II. Occurrence.

The material for the following investigation was obtained from moles caught on different farms in mid and north Cardiganshire during the months from December, 1930 to March, 1931.

(*a*) *Ityogonimus lorum.*

This species is fairly widely distributed throughout Europe. It was reported by Dujardin from the neighbourhood of Cannes, by Looss in Germany, and Stossich also refers to it as occurring in Denmark and Austria. Dujardin observed that out of 74 moles examined, 28 or 37·8 per cent. were parasitised by this species.

In the present investigation, of the 201 moles examined 37 or 18·4 per cent. were parasitised. The number in each host varied from 1 to 11, the average being 3·3. There seemed to be slight increase in the number infected in February and March. The number examined at this period, however, is too small to suggest a seasonal variation.

(*b*) *Ityogonimus talpae.*

Dujardin recorded this parasite as occurring in the north-west of France, Müller in Denmark and all the other authors in Germany. Zeder refers to this species being found most often in winter, rarely in autumn and spring and never in summer.

Of the 201 moles examined during the present observations, 48 or 23·8 per cent. were parasitised; the number in each individual host varied from 2 to 6.

Dujardin (1845), in his account of this species, refers to Müller's work, pointing out that of 382 moles examined by the latter, 68 or 17·8 per cent. were infested.

Gonder (1910) investigated a number of moles from the neighbourhood of Giessen and Frankfurt and pointed out that those infested with *Ityogonimus talpae* were caught on damp meadows and that those caught on dry land were not infested, and states that perhaps the reason for this was the presence of a specific intermediate host in the locality concerned. The moles examined during the present investigation were caught in both wet and dry land and the highest infestation was noticed in moles obtained from fertile wet alluvial soil.

Gonder gives a full description of this species, and as far as can be seen agrees with that of the writer except in the dimensions of the body. Gonder's specimens were 8–11 mm. long by 0·25–0·7 mm. broad, whereas those in the present investigation were 19–30 mm. long by 0·3–1·0 mm. broad.

III. ANATOMY OF *ITYOGONIMUS LORUM*.

(*a*) *External characters.*

The body is long and cylindrical in shape (Fig. 1). It varies in length from 3·8 mm. to 10·5 mm. (average 5·9 mm.). The breadth is fairly regular throughout, being slightly greater in the region of the ventral sucker where it varies from 0·42 mm. to 1·0 mm. (average 0·66 mm.).

The body is more or less of uniform thickness throughout. In a specimen 5 mm. long the depth at the anterior end is 0·2–0·25 mm.

The oral sucker, 0·22–0·47 mm. in diameter, is situated subterminally on the ventral surface of the anterior end (Figs. 1 and 2), the distance between the two suckers being equivalent to one-third or one-fourth of the body length. The ventral sucker measures 0·32–0·67 mm.

The body surface is covered with an unarmed cuticle, thrown into transverse folds in the contracted animal.

Both suckers are well developed, the arrangement of the muscles being almost identical with that described for other trematodes.

The parenchyma exhibits a difference in consistency in different parts of the body. At the anterior end of the worm it is fairly compact, towards the middle of the body it is vacuolated, but becomes more compact again towards the posterior extremity.

(*b*) *Alimentary canal.*

The mouth lies in the centre of the oral sucker and leads through a distinct prepharynx (Fig. 2). The pharynx is 0·08–0·15 mm. long by 0·13 mm. broad. Histologically it is very similar in structure to the suckers, there being a layer of transverse muscles beneath the cuticle both externally and internally. The main body of the pharynx consists of radiating muscle fibres by whose contraction the lumen is enlarged during sucking. The triangular pharyngeal cavity leads to a short oesophagus 0·07–0·15 mm. long with the lumen 0·02–0·025 mm. in diameter. The oesophagus bifurcates posteriorly into two intestinal branches. These pass dorsally over the genital organs and each inclines slightly inward, where it ends blindly behind the posterior testis. The intestinal limbs are devoid of caeca. The intestinal wall consists of a single layer of columnar epithelium.

Numerous cells were observed around the posterior portion of the pharynx and the anterior portion of the oesophagus. The ducts of these glands could not be traced. Gonder (1910) refers to them in *Ityogonimus talpae* and suggests for them a digestive function.

(*c*) *Excretory system.*

The excretory pore (Fig. 4) lies somewhat at the posterior extremity. The excretory vesicle opening by this pore, is very short and bifurcates behind the

branches of the intestinal limbs, the two main branches passing externally and ventrally to the intestine. In the pharyngeal region each excretory duct reflexes upon itself, forming a loop. The returning ducts could not be traced further than the level of the oesophagus. As it was not possible to study living material, none of the flame cells could be made out.

(*d*) *Genital organs.*

(1) *Male.* The two testes, oval in outline, lie in the posterior region of the body (Figs. 4 and 5). The anterior testis measures 0·24 × 0·17 mm. The posterior testis lies 1·4 mm. behind the anterior and is slightly the larger, being 0·3 mm. × 0·19 mm. The anterior vas deferens originates on the posterior lateral margin of the anterior testis, while the posterior vas deferens arises from the anterior margin of the posterior testis whence it runs forwards. The two vasa deferentia meet just behind the ovary to form the unpaired vesicula seminalis which is continued posteriorly to open by the genital aperture in the neighbourhood of the posterior testis. The vesicula seminalis is a coiled tube about the diameter of the uterus. Posteriorly the vesicula seminalis communicates through the pars prostatica with the cirrus. The pars prostatica is surrounded by a number of small club-shaped prostatic glands; it suddenly narrows as it passes into the ductus ejaculatorius. The cirrus is bell-shaped, has a highly muscular wall and opens on a slight protrusion by the genital aperture (Fig. 4).

(2) *Female.* The unpaired ovary (Figs. 4 and 5) is spherical, 0·15–0·17 mm. in diameter and lies between the two testes. Arising from the postero-dorsal margin is a short slender oviduct, 0·075–0·1 mm. long, running a straight course and opening behind into a fusiform shell gland 0·075–0·15 mm. in its greatest diameter.

The vitelline glands (Fig. 1) extend from behind the ventral sucker along the lateral parts of the body as far back as the anterior testis. They consist of a cluster of unicellular cells each giving off a delicate duct. The two main vitelline ducts extend on each side of the body, gradually approaching each other between the anterior testis and the ovary, and a short distance behind the latter they turn in rapidly and unite to form a conspicuous triangular swelling—the yolk reservoir (Figs. 4 and 5). From this originates the median vitelline duct 0·05–0·08 mm. long which runs directly dorsally and opens into the distal portion of the oviduct. Laurer's canal was only observed in one specimen; it opens into the oviduct on the dorsal side, a little anterior to the vitelline duct. From the oviduct it takes a straight course to open to the exterior in the mid dorsal line 1·17 mm. in front of the posterior extremity in a specimen 6·45 mm. long. The canal in this case was 0·15 mm. long. There is no receptaculum seminis as already observed by Looss (1907).

The cavity of the shell gland is continuous behind with the uterus, which runs posteriorly for a short distance, loops ventrally and pursues a winding course forward, usually to about midway between the posterior border of the

ventral sucker and the anterior termination of the yolk glands. This, however, seems to vary, as sometimes it almost touches the former and in other specimens it extends only to the termination of the yolk glands. It loops round again ventrally taking a winding course posteriorly and opens side by side with the male genital aperture. In mature specimens, the uterus is filled with ova measuring 0·03–0·035 mm. by 0·015–0·017 mm.

IV. SYSTEMATIC POSITION.

The genus *Ityogonimus*, which belongs to the family Harmostomidae, is characterised by (*a*) the unarmed cuticle, and (*b*) the uterus does not extend in front of the ventral sucker. The specific characters of *I. lorum* are: (*a*) the absence of a receptaculum seminis, (*b*) the restriction of the vitelline glands to the region behind the ventral sucker, and (*c*) the ventral sucker being well developed.

The synonymy of the two species of *Ityogonimus* which occur in the mole is confused through the same species having been described under different names and different species under the same name. *I. lorum* has been described by Dujardin (1845), Stossich (1892) and Looss (1907), the latter referring to it as *I. filum*. Table I gives the measurements in mm. of *I. lorum* as recorded by previous authors and by me.

Table I.

Measurements in mm.

	Dujardin	Stossich	Looss	Writer
Length	8–30	8–34	4·5–5·0	3·8–10·75
Breadth	0·5–0·8	0·3–0·1	0·4–0·48	0·42–1·0
Oral sucker	0·25–0·48	—	0·28	0·22–0·47
Ventral sucker	0·45–0·48	Bigger than oral sucker	0·38	0·32–0·67
Distance between suckers	2·65–3·0	—	—	1·10–3·50
Testis	Oval	Oval	Round	Oval
Eggs	0·03–0·33 × 0·015	0·33 × 0·015	0·029–0·032 × 0·013–0·015	0·03–0·035 × 0·015–0·017

Looss' description of the internal organisation agrees with my observations except for his statement that the anterior bending of the uterus lies usually as far back from the front end of the vitelline glands as the latter do behind the ventral sucker. In my specimens the reverse was generally the case, the vitelline glands lie as far back from the anterior bending of the uterus as the latter does behind the ventral sucker. This seems to vary, however, as already indicated, but the vitelline glands were not observed to extend in front of the uterus.

The other species, *Ityogonimus talpae*, was described by Dujardin (1845) as *Monostomum ocreatum* and by Gonder (1910), who gave a full description under the name *Ityogonium lorum*.

In comparing the two species of *Ityogonimus*, the essential differences are: (*a*) the comparative size of the oral and ventral suckers, and (*b*) the

relative extent of the yolk glands as pointed out by Dujardin (1845), (*c*) the absence of receptaculum seminis in *I. lorum*. I may add that the U-shaped arrangement of the vesicula seminalis, as described by Gonder in *I. talpae*, was not observed by me in *I. lorum*.

The body measurements in both species vary considerably as shown in Table II.

Table II.

Measurements in mm.

	I. lorum		*I. talpae*	
	Length	Width	Length	Width
Dujardin	8 –30	0·5 –0·8	15–54	$\frac{1}{30}$–$\frac{1}{20}$ of length
Stossich	8 –34	0·3 –1·0	—	—
Looss	4·5– 5	0·4 –0·48	—	—
Gonder	—	—	8–16	0·25–0·7
Davies	3·8–10·57	0·42–1·0	19–30	0·30–1·0

V. Conclusions.

Both species of *Ityogonimus* are fairly prevalent in moles in the Aberystwyth district, the percentage of infestation and degree of parasitisation with *I. talpae* being slightly higher than with *I. lorum*. As observed by Gonder (1910), *I. talpae* occurs more commonly in moles caught in damp soils.

My observations regarding the synonymy support Witenberg's views. *I. filum* (Looss 1907) is synonymus with *I. lorum* (Duj. 1845); *I. lorum* (Gonder 1910) and *Distoma ocreatum* (Duj. 1845) are synonyms of *I. talpae* (Goeze, 1872).

Note. The occurrence of other trematode parasites of the alimentary canal has been noted. *Omphalomenta flexuosa* (Rud. 1809) was found on five occasions in the small intestine of moles obtained from one of the farms. In these instances not more than two were found in the same host.

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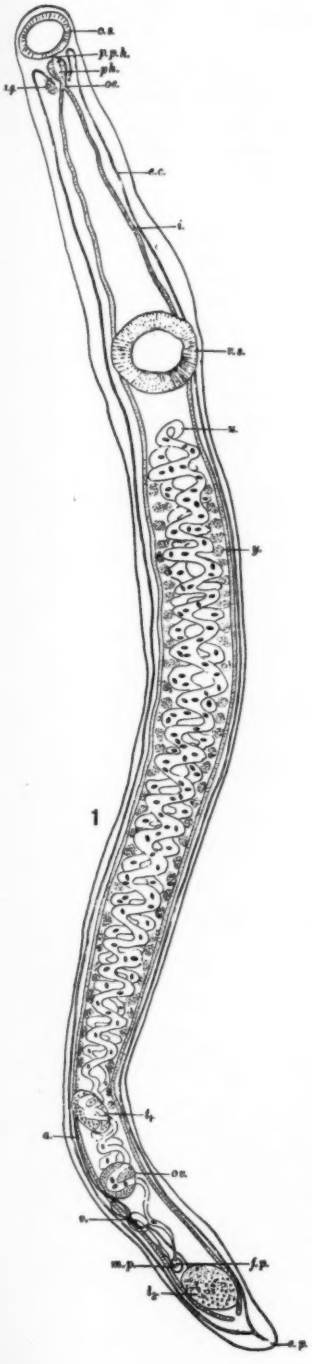
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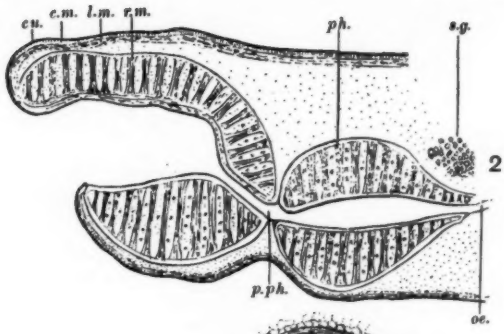
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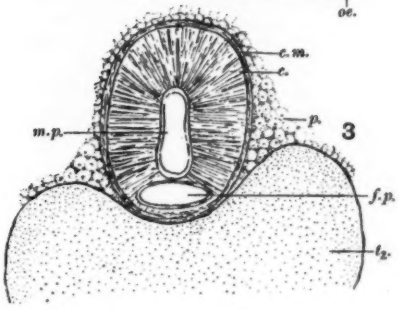
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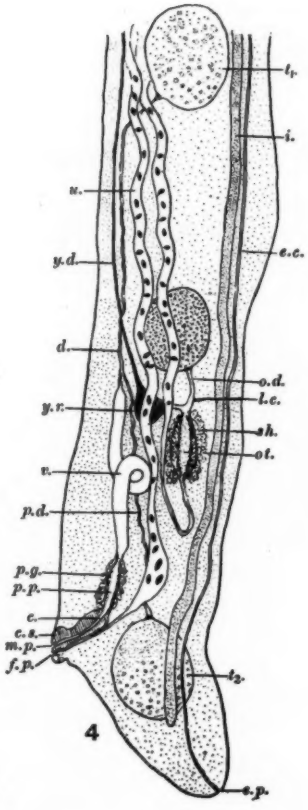
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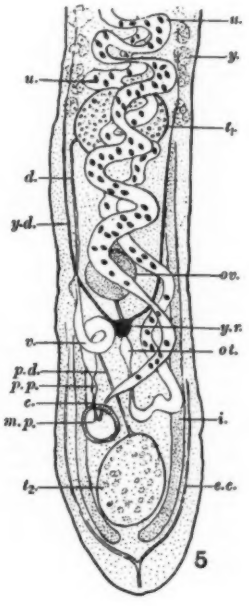
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DESCRIPTION OF PLATE XII

Ityogonimus lorum (Duj. 1845).

Fig. 1. General outline.
Fig. 2. Longitudinal section of oral sucker oesophagus and pharynx.
Fig. 2. Transverse section through genital aperture.
Fig. 4. Genitalia (lateral view).
Fig. 5. Genitalia (ventral view).

Lettering.

a.	anterior vas deferens	*p.*	parenchyma
c.	cirrus	*p.d.*	posterior vas deferens
c.m.	circular muscles	*p.g.*	prostrate glands
c.s.	cirrus sac	*ph.*	pharynx
cu.	cuticle	*p.p.*	pars prostatica
d.	anterior vas deferens	*p.ph.*	prepharynx
e.c.	excretory canal	*r.m.*	radial muscles
e.p.	excretory pore	*s.g.*	salivary glands
f.p.	female genital pore	*sh.*	shell glands
i.	intestine	t_1.	anterior testis
l.c.	Laurer's canal	t_2.	posterior testis
l.m.	longitudinal muscles	*u.*	uterus
m.p.	male genital pore	*v.*	vesicula seminalis
o.d.	oviduct	*v.s.*	ventral sucker
oe.	oesophagus	*y.*	yolk glands
o.s.	oral sucker	*y.d.*	yolk duct
ot.	ootype	*y.r.*	yolk reservoir
ov.	ovary		

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ON THE SPOROCYST AND CERCARIA OF A MARINE DISTOMID TREMATODE FROM *NUCULA*.

By E. IDRIS JONES, B.Sc. (London)
and MIRIAM ROTHSCHILD.

(With 5 figures in the Text.)

In May, 1931, 2 per cent. of the *Nucula nucleus* dredged from the Mewstone grounds at Plymouth (25–30 fathoms) were found to be infected with a hitherto undescribed sporocyst and cercaria. Between June and September, 800 *N. nucleus* were obtained from the same locality, and one in sixty contained the parasite.

Technique.

The sporocysts and cercariae were studied alive in sea water and normal saline. The best preparations were obtained with material fixed in Carnoy's fluid or Bouin-Duboscq, and stained with borax carmine or Delafield's haematoxylin.

The sporocyst (Fig. 1).

Infected specimens of *Nucula* can be identified by the opaque whiteness of the visceral mass due to the presence of the parasite. The visceral mass is covered by a thin membrane which encloses the sporocysts. The gonad is generally completely destroyed, and the parasites are found lying between the digestive diverticula. About 50–120 sporocysts occur in each infected host.

The sporocysts are elongated unbranched tubes of uniform diameter, 1·7–2·5 mm. long and 0·15–0·28 mm. wide. They are covered with a fine unarmed cuticle, and are capable of considerable elongation and contraction, the cuticle being thrown into transverse striations during contraction. The only organ seen in the sporocyst is a terminally situated birth pore, through which the full-grown cercariae emerge.

The cercaria (Figs. 2 and 3).

General form. The body is spindle-shaped and carries a forked tail at the posterior end. A large ventral sucker is situated in the middle line and a smaller sucker, which is not quite terminal but placed ventrally, is present at the anterior end.

The tail consists of a very short stem piece, from which arise the two furcae which are three or four times the length of the body and are capable of great extension and contraction. In section they are seen to possess well defined

longitudinal muscle fibres. Circular muscle fibres are present at their proximal ends, but cannot be clearly distinguished distally.

The entire animal is covered by a spineless cuticle.

Superficially the cercaria somewhat resembles a *Bucephalus*.

The measurements in *microns* are given below:

Body-length, when expanded	460–580
,, ,, contracted	250
Body-width at level of ventral sucker	133
Furcae, length of common stem	16·6–25
Furcae, length when extended	924–1540
Furcae, average width	10
Ventral sucker, diameter	93–112
Anterior sucker, diameter	45 × 43–79 × 66
Pharynx, diameter	43 × 46
Oesophagus, length	98

The alimentary canal (Fig. 2). The mouth is surrounded by the anterior sucker. A short muscular pharynx lies immediately behind it and leads into a long oesophagus about twice the length of the pharynx. Prepharynx absent. The oesophagus terminates in a ∩-shaped gut, the limbs of which pass dorsally on either side of the ventral sucker.

The excretory system (Fig. 3). There are twenty-three flame cells on each side of the body, which are connected with the excretory canals by fine canaliculi. Each canal originates near the posterior end of the body and passes anteriorly to the level of the pharynx, following the course of the limb of the excretory vesicle, and sometimes lying dorsal to it. Here it bends sharply backwards and proceeds posteriorly for a short distance before entering the apex of the excretory vesicle, at about the level of the pharynx.

The limbs of the vesicle pass downwards on either side of the ventral sucker. They unite to form a common stem at the posterior end of the body which then passes into the common tail-piece by a very narrow canal. It then divides again and each branch continues to the distal end of one of the furcae, where it opens to the exterior by a minute pore. The portion of the excretory vesicle in the body is packed with dark granules which help to display the typical lyre-shape of the system. The granules exhibit a streaming motion but only pass singly or in twos into the furcae.

The system is most clearly seen immediately before death. The cilia of the flame cells can then be observed beating actively.

The reproductive system (Fig. 3). In whole mounts of the cercaria, stained with Delafield's haematoxylin, two small triangular masses of nuclei are visible, one immediately behind and one in front of the ventral sucker. These probably represent the rudiments of the gonads and cirrus sac respectively.

Locomotion. It is unlikely that the cercariae employ their furcae actively as locomotor organs, but they probably help in keeping them afloat in the water. If they sink to the bottom of the dish in which they are examined, a leech-like mode of progression is sometimes employed. The body of the cercaria is elongated, the anterior sucker pressed on the substratum and the body drawn forward by a subsequent contraction.

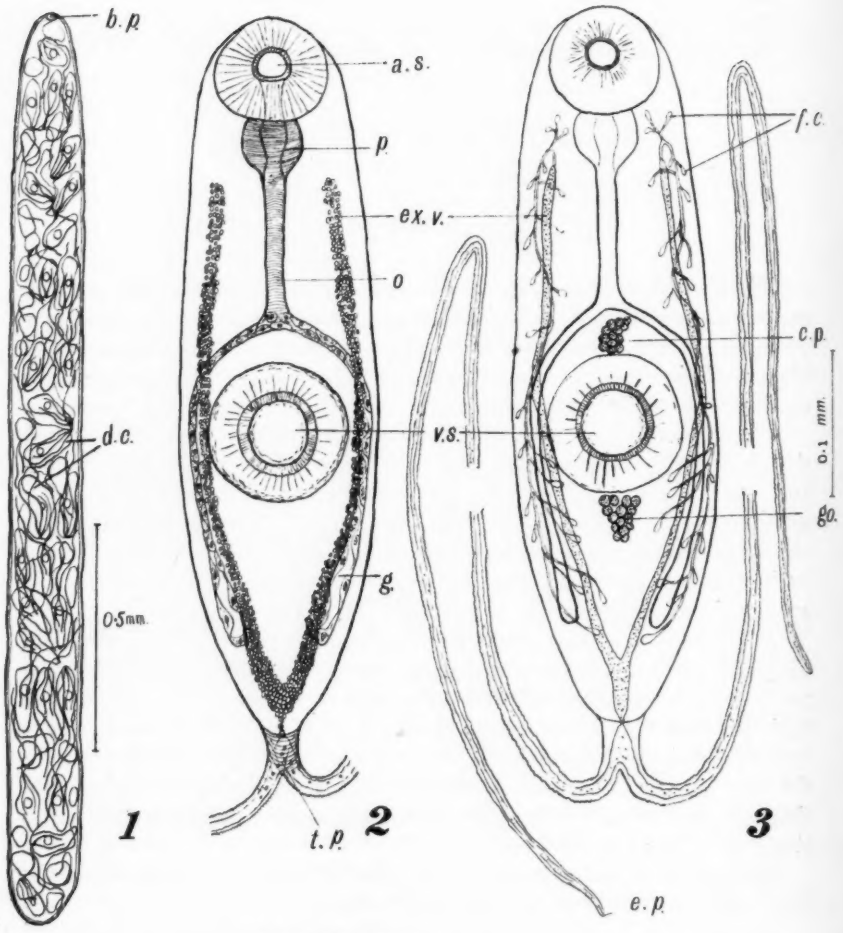

Fig. 1. Sporocyst.
Fig. 2. Cercaria.
Fig. 3. Cercaria—excretory system.

Lettering: *a.s.* anterior sucker.—*b.f.* bud of furca.—*b.p.* birth pore.—*c.p.* cirrus-pouch(?).—*d.c.* developing cercariae.—*e.p.* excretory pore.—*ex.v.* excretory vesicle.—*f.c.* flame cells.—*g.* limb of gut.—*go.* gonad (?).—*o.* oesophagus.—*p.* pharynx.—*t.p.* tail-piece.—*v.s.* ventral sucker.

The development of the excretory vesicle (Figs. 4 and 5). All stages in the development of the cercaria from the germ ball to the full grown condition are found within the sporocysts. The excretory system is first evident as a pair of short tubes in the distal ends of the furcae. They gradually elongate until they extend the entire length of the furcae and into the body of the embryo to the level of the ventral sucker. At this stage they are distinct in the common tail stem, but are united for a short distance at the posterior part of the body. In later stages the tubes fuse in the tail-piece and extend further anteriorly.

We were unable to trace the development of the flame cells.

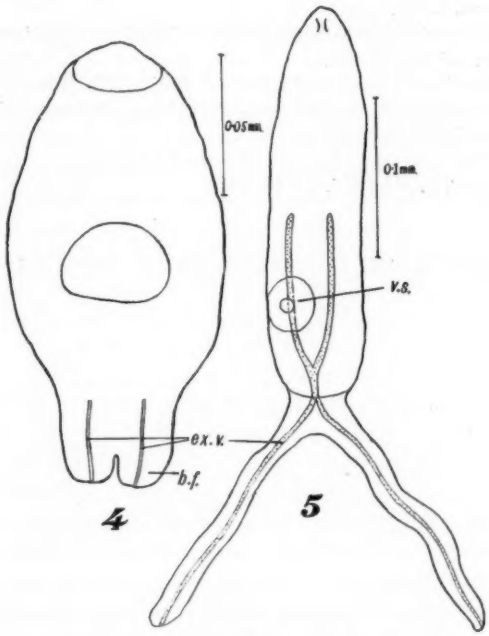

Fig. 4. Development of excretory vesicle, early stage.
Fig. 5. Development of excretory vesicle, later stage.
For lettering see Figs. 1–3.

SUMMARY.

The cercaria is liberated from an unbranched sporocyst by the birth-pore. It is spindle-shaped and possesses a bifid tail. It possesses two suckers, a long oesophagus and a forked gut. The excretory vesicle is Y-shaped with two furcal extensions from its stem. The cuticle is unarmed. There is no stylet or cystogenous organ.

There is no redia.

All stages in the development of the cercaria are found within the sporocyst.

ACKNOWLEDGMENTS.

We are deeply indebted to Dr E. J. Allen, F.R.S., for the facilities he afforded us for working at the Laboratory at Plymouth and to Dr M. Lebour, Mr W. H. Leigh-Sharpe and Mr F. J. Brown for their help and advice.

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OESOPHAGOSTOMUM MULTIFOLIATUM N.SP.

AN UNDESCRIBED NEMATODE FROM SHEEP AND GOATS.

By R. DAUBNEY and J. R. HUDSON.

Division of Veterinary Research, Kenya Colony.

(With 5 Figures in the Text.)

The species of *Oesophagostomum* here described has been collected quite frequently from sheep and goats in certain parts of the colony, always accompanied by the commoner oesophagostome *O. columbianum*.

The inflation of the cephalic vesicle draws attention to the presence of the species when mixed lots are examined under the binocular microscope in the fresh state. The size of the eggs (up to 0·16 mm. long) found in the faeces is also sufficient to cause a special search to be made for the parent worm; particularly since *Nematodirus*, the only strongyle genus whose eggs attain such dimensions, has not hitherto been recorded from Kenya.

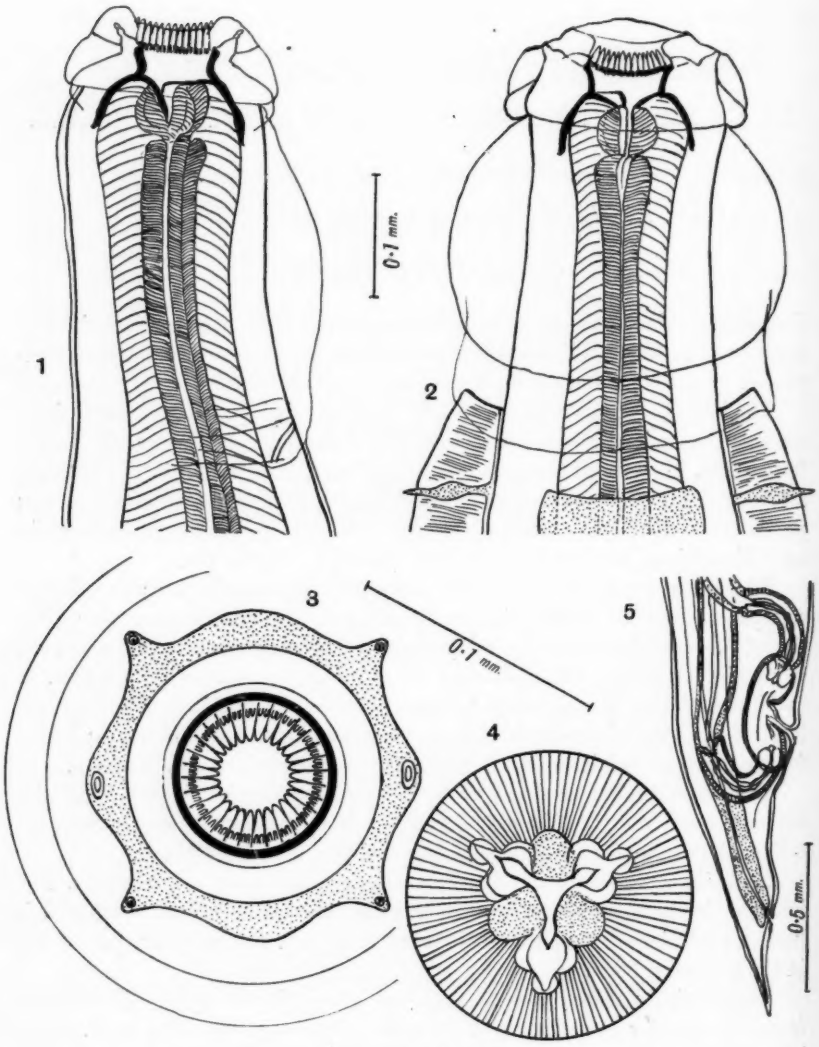
The worms are pure white, bent ventrally at the anterior end; they do not straighten out after fixation in hot 70 per cent. alcohol. They are about the size of *O. columbianum*, the females being 14–17 mm. long and up to 0·48 mm. in maximum width, the males are 12–14 mm. long and up to 0·4 mm. in maximum width. Well-developed lateral alae are present, extending from the level of the ventral groove almost to the posterior end of the worm; in the female the alae terminate in the region between the vulva and the anus.

The mouth collar (marked off by a distinct groove) is in the form of a truncate cone, and the circum-oral papillae do not project distinctly from the collar; the sub-median papillae are more slender than the laterals.

Cephalic vesicle well developed, extending backward 0·2–0·28 mm. The inflation is confined to the ventral and lateral aspects of the worm and is limited posteriorly by the transverse ventral groove. The excretory pore opens in the ventral groove.

The cervical papillae pass through the lateral alae at about 0·37–0·4 mm. from the anterior extremity and the nerve ring lies at the same level. Each lateral ala for the greater part of its extent is roughly equal in breadth to one-fourth of the actual thickness of the body.

The mouth opening is circular. The external leaf consists of thirty long triangular elements which turn outward at their tips. The internal leaf-crown contains sixty short, almost rectangular elements, two at the base of each leaf of the external crown. The wall of the buccal capsule converges a little in its posterior third, but finally tends to diverge.



Figs. 1–5.

Fig. 1. ♂, anterior end, lateral view.
Fig. 2. ♀, anterior end, ventral view.
Fig. 3. ♀, anterior end, *en face*, semi-diagrammatic, part of circular contour omitted.
Fig. 4. Oesophageal funnel in optical section, *en face*.
Fig. 5. ♀, not fully mature, posterior end.
Figs. 1 and 2 drawn to the same scale; Figs. 3 and 4 also to one scale.

The oesophageal funnel is fairly well developed, although its lumen usually appears to be small. The cuticular lining of the funnel, however, is greatly thickened, and is ridged externally where the muscles are attached. Throughout the oesophagus, which is 0·9–1·0 mm. long, the cuticular portion constitutes between one-third and one-half of the total thickness of the oesophagus. The cuticular thickening of the oesophageal funnel is roughly cup-shaped and quite definitely separated by a sharp constriction from the remainder of the oesophageal cuticle. Fig. 4, which represents an optical section of the oesophageal funnel viewed *en face*, gives an idea of the elaborate reinforcement of the cuticular lining.

The bursa of the male is indistinguishable from that of *O. columbianum*; the rays, spicules, accessory piece and genital cone have the same form and size as in that species.

The tail of the female is shorter than that of *O. columbianum*, and tapers finely at the tip. The vulva is also situated more posteriorly. The distance from the anus to the tip of the tail is 0·26–0·3 mm. and the vulva is 0·55–0·6 mm. in front of the anus. In gravid females the lips of the vulva are more prominent than in the specimen shown in Fig. 5. The vagina is extremely short and is transversely directed. The ovejectors are similar in form to those of *O. columbianum*, the *pars ejectrix* being roughly 0·3 mm. long. The eggs in the uterus measure 0·15–0·165 × 0·068–0·08 mm.

Hosts. Sheep and goats.

Habitat. Large intestine.

Locality. Kenya Colony.

The following short key to the species described from domestic ruminants may assist in determination:

1. Lateral alae absent. Vagina directed anteriorly, longer than *pars ejectrix* of ovejectors. Cervical papillae post-oesophageal. **2**

Lateral alae present. Vagina directed almost transversely, extremely short. Cervical papillae situated anteriorly to the middle of the oesophagus. **3**

2. Elements of external leaf-crown 12 in number. Spicules 1·5–1·7 mm. long. *O. asperum.*

Elements of external leaf-crown 18 in number. Spicules 1·1–1·2 mm. long. *O. venulosum.*

3. Cephalic vesicle not inflated. Elements of external leaf-crown 18 to 24 in number. *O. columbianum.*

Cephalic vesicle inflated. **4**

4. External leaf-crown absent. Eggs 0·075–0·085 × 0·04–0·045 mm. *O. radiatum.*

External leaf-crown present, consisting of 30 elements. Eggs 0·15–0·165 × 0·068–0·08 mm. *O. multifoliatum.*

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A NEW NEMATODE, *TYLENCHUS APTINI* N.SP., PARASITE OF THYSANOPTERA (INSECTA: *APTINOTHRIPS RUFUS* GMELIN).

By U. S. SHARGA, M.Sc., Ph.D. (Edin.).

From the Department of Agricultural Zoology, University of Edinburgh.

(With 26 Figures and a Chart.)

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I. Introduction.

The study of *Aptinothrips rufus* made in 1930, in the vicinity of Edinburgh, revealed the presence of a nematode parasite in the insect which seemed worthy of investigation. Little is known of the parasites of Thysanoptera in general, and I can find no record of any parasite of *Aptinothrips*.

Uzel (1895) described a nematode in *Thrips physopus*, and P. R. Jones, in 1910 (Russell, 1912), found a nematode "working in the full-grown larvae of the bean thrips," *Heliothrips fasciatus*, at Lindsay, California. In the absence of any description or figures of either of these species, they cannot be identified with the nematode described in this paper.

The nematode infesting *Aptinothrips* was first found in December, 1929, and subsequently a regular record of the parasitised thrips was kept to find out the degree of parasitism in various months of the year, and thrips from various localities were examined to determine the distribution of the parasite. The morphology and bionomics were studied, but further work on the free-living stages is required.

Eggs, larvae and females were generally found and sometimes only eggs

and females. Two morphologically different larvae were noticed, (*a*) having a conical tail and stylet, and (*b*) the male having no recognisable buccal stylet, but possessing caudal alae, spicula and gubernaculum. The younger and maturer stages of the two forms and all the stages in the development of the parasitic female occurred together—though rarely. The early stages of the parasitic female were quite unlike the mature females.

The host *Aptinothrips rufus* has two varieties, one with six- and one with eight-jointed antennae and although the six- and eight-jointed forms were sometimes found together (eight-jointed very scarce) in the infested locality, yet in no case so far have the worms been taken from the body cavity of the eight-jointed form. The infection also has so far been found in female thrips only. Males are, however, very scarce in this species, and only four males, all uninfected, were captured from the infested areas in the whole year. The infection was found to be greatest in adult females; it was rare in larvae and pupae, being only observed in a few cases.

My observations were carried out in the Department of Agricultural Zoology, University of Edinburgh, under the supervision of Dr C. B. Williams, to whom I am greatly indebted for many valuable suggestions and advice. I also have to thank Dr H. A. Baylis for some important suggestions.

II. TECHNIQUE.

The insects were dissected alive in water. In the case of infected specimens, eggs, larvae, males and females of the nematode came out immediately. The worms were studied after being killed on a slide over a heated plate for a short time. In order to slightly colour the worms and their internal organs, a trace of 1 per cent. aqueous solution of Nile blue was added to the drop of water containing the nematodes.

To preserve specimens of nematodes, Lactophenol (Amann, 1896), suggested by Dr H. A. Baylis, was used and found very satisfactory. Its composition is as follows:

Carbolic acid	20 grm.
Syrup lactic acid	20 ,,
Glycerine	40 ,,
Distilled water	20 ,,

The specimens were mounted directly in the above medium after being killed by heat, the coverslip being sealed with asphalt or gold size. The internal structures of the mature females were very clearly seen.

A second method used was to fix the worms in a drop of Bouin's fluid and stain them with Delafield haematoxylin and eosin. The reproductive organs of normal and parasitised thrips were also studied by this method.

III. SYSTEMATIC POSITION.

The systematic position of the worm parasitic in *Aptinothrips* has to be determined from the stages found in the host's body cavity, *i.e.* the egg, early

and advanced stages of the first form larvae, mature and immature stages of males (second form) and various stages in the development of parasitic female.

I am provisionally regarding the worm as a highly modified member of the genus Tylenchus because of its behaviour and morphological characters and give it the specific name "*aptini*."

Recently, Goodey (1930) established a genus *Tylenchinema* for the reception of *Tylenchinema oscinellae* n.sp. parasitic in the frit-fly (Diptera, Cecidomyidae). The parasitic female is a large coiled sausage-shaped nematode quite different from the thrips parasite.

The genera Aphelenchus and Heterodera include plant parasitic species, whose characters are very different from this species.

Fuchs (1915) described *Tylenchus contortus* and *T. dispar* from *Ips typographus* and *Cryphalus picae* (Coleoptera, Scolytidae), making two varieties of the former and three varieties of the latter. Wülker (1923) described a third species *Tylenchus hylastis* from *Hylastis ateri* (Coleoptera, Scolytidae).

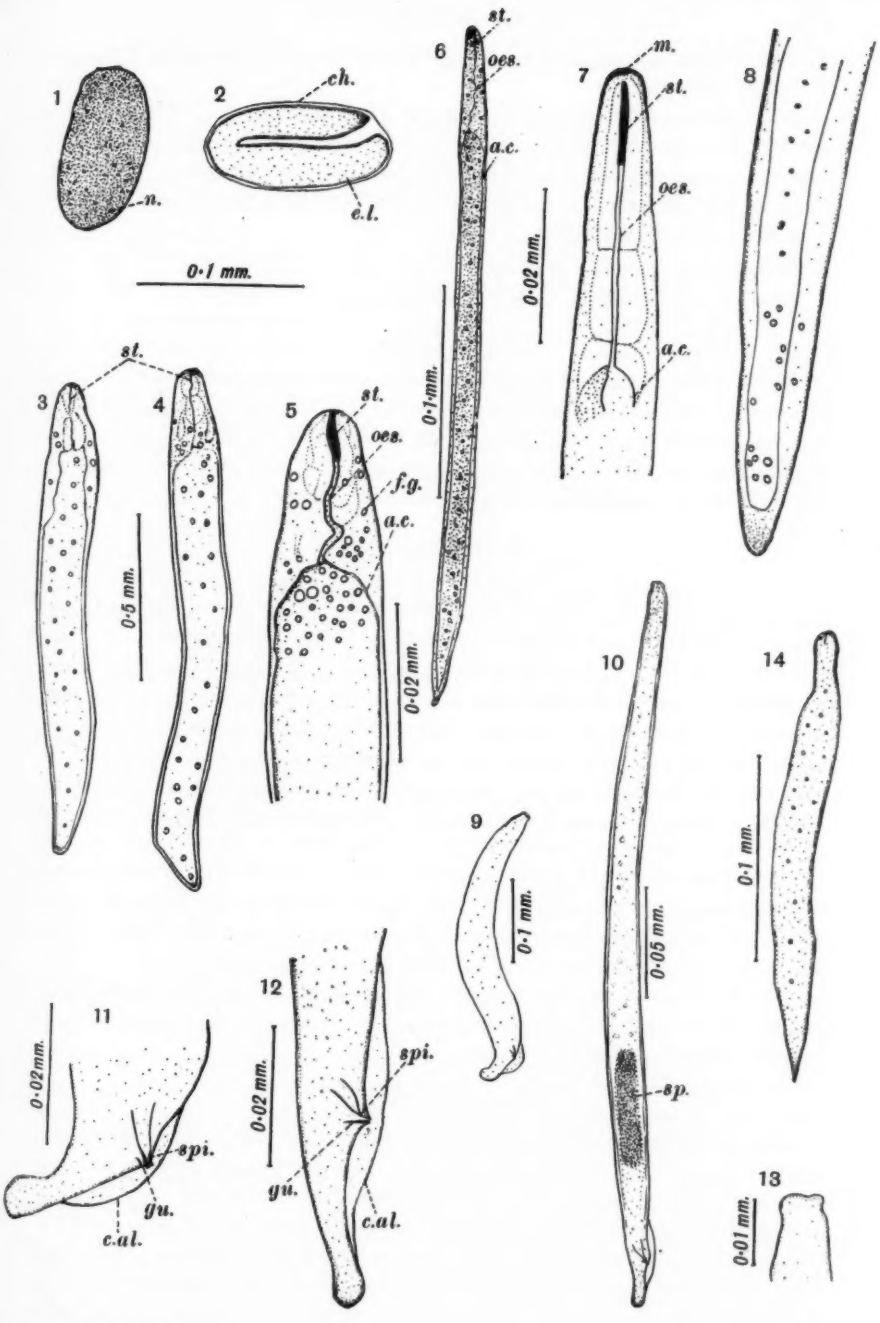
The parasitic female, as described and figured by Fuchs (1915, Pl. 17, figs. 12–13), resembles the early stages of the parasitic female from *Aptinothrips* illustrated in Figs. 16–18. There is, however, a much greater morphological and biological similarity between the parasitic female of *Tylenchus dispar curvidentis* (Fuchs (1915*a*), p. 196, fig. 1) and the parasitic stages of this female (Figs. 20–21). The presence of a stylet, trace of oesophagus, constitution of ovary, oviparity, uterus and position of vulva are, among others, common characters. *Tylenchus aptini* can be separated from Allantonema, Bradynema and Howardula chiefly on the basis of the above-mentioned characters, of which the presence of a stylet in the early stages and a trace of it in degenerated later stages are the characters common to other forms of Tylenchus. If the second form of worm with caudal alae, spicula and gubernaculum be taken as

Explanation of Text-figs. 1–14.

Tylenchus aptini.

Fig. 1. Egg from host's body cavity.
Fig. 2. Young larva inside the egg.
Fig. 3. Young larva (*form* 1) after emerging from egg.
Fig. 4. Slightly advanced stage of larva (*form* 1).
Fig. 5. Anterior part of same larva showing stylet, oesophagus and intestine.
Fig. 6. Mature larva (*form* 1) from host's body cavity.
Fig. 7. Anterior end of the same with a stylet, oesophagus and an alimentary canal.
Fig. 8. Hind part of the same.
Fig. 9. Young stage of male (*form* 2) with caudal alae, spicula, etc.
Fig. 10. Mature stage of male (*form* 2) with caudal alae, gubernaculum and a pair of spicules.
Fig. 11. Hind part of the young stage of male (*form* 2).
Fig. 12. Hind part of mature male.
Fig. 13. Anterior part of the male showing constriction.
Fig. 14. Early stage of the parasitic female from host's body cavity.

Lettering: *a.c.* alimentary canal; *c.al.* caudal ala; *ch.* chorion; *e.* egg; *e.l.* ensheathed larva; *f.g.* fat globules; *gu.* gubernaculum; *m.* mouth; *n.* nuclei; *oes.* oesophagus; *o.s.* old skin; *o.t.* ovarian tube; *ov.* ova; *re.m.* reserve mass; *st.* stylet; *sp.* spermarium; *spi.* spiculum; *t.p.* terminal papilla; *ut.* uterus; *ut.w.* uterine wall; *v.* vulva.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the male of this species, which is very likely, the relationship with Tylenchus is further enhanced.

Since the degeneration of the mature parasitic female of this species is much greater than hitherto known in insect-infesting Tylenchus, and the other characters are so different in known species of Tylenchus, it is provisionally regarded as a new species until the true relationship of the free-living stages may yield more information.

IV. Description.

(i) *Eggs.*

The eggs (Fig. 1) are oval, broadly rounded anteriorly and posteriorly, and measure 40–60 × 20–28μ, and have a thin smooth chorion. They lie free in the host's body cavity. During growth, the protoplasm shrinks inside from the chorion. The young larvae (Fig. 2) develop inside the eggs, ultimately rupture the shell and emerge in the coelomic fluid of the host.

(ii) *Larvae* (*Form* 1).

The young larvae on emergence (Figs. 3 and 4) measure 120–140 × 13–17μ, the head at the middle of stylet is 10μ broad. They are rounded anteriorly and have a conical tail. The mouth is followed by a stout stylet connected with a thin winding oesophagus which opens into a wide, long alimentary canal occupying the rest of the body (Fig. 5). Large fat globules staining deeply with Nile blue are found in the intestine. Some clear globular areas are seen beside the stylet. After several ecdyses the larvae become longer and thinner.

The older larvae (Figs. 6, 7 and 8) are found in large numbers. They are 250–366μ long, 13–17μ wide (maximum) tapering anteriorly to 3–7μ and to 3μ at the tail which ends in a sharp point. The head is rounded and smooth (Fig. 6). Separate lips or papillae have not been observed. The general outline of these mature larvae resembles some figures of Wülker (1923, pp. 401, 404, 410) of the development of Allantonema larvae and figures of Fuchs (1915, Pl. 18, fig. 18*a*) of the larva of *Tylenchus dispar typographi*.

The larvae obviously feed on the fluid contents of the host's abdominal cavity. The young and advanced larvae, but for their size, are similar in essential morphological characters.

(iii) *Male* (*Form* 2).

The second form differs morphologically from the first, both in young and mature stages. They are 131μ long by 17μ (greatest width); distance from tip of head to gubernaculum 120μ, from gubernaculum to tail end 11μ.

These young larvae (Fig. 9) are very few in some dissections. They are distinguished by their peculiar behaviour and by their morphology. The head is flat anteriorly and is separated from the main body by a slight constriction. No distinct mouth or stylet is seen, but a degenerate oesophagus is present.

A pair of caudal alae, spicules and a very minute gubernaculum are present (Fig. 11).

After several moults the larvae assume the mature male form (Fig. 10); they are 248–333μ long with the greatest width 13–20μ; distance from anterior end to gubernaculum 291μ, from gubernaculum to tail end 21–27μ.

The body tapers at both ends. The head is flat, broader than the tail, and separated from the body by a slight constriction (Fig. 13). The tail is bluntly rounded. Lips or papillae are absent. The mouth aperture is not visible, there is no trace of a stylet or oesophageal bulb and no intestinal gland is visible.

The tail is curved posteriorly, constricted with end rounded (Fig. 12). On each side of the tail end are cuticular expansions or caudal alae which arise a little anterior to the anus from the ventral edge of the lateral field, and almost attain the tail end; the free edges of the alae are not crenated.

The spicules are broad anteriorly and tapering posteriorly towards the tip. A short gubernaculum is also present. The spermaria consist of a long glandular area applied to the side of the intestine. In all these characters it resembles many species of Tylenchus.

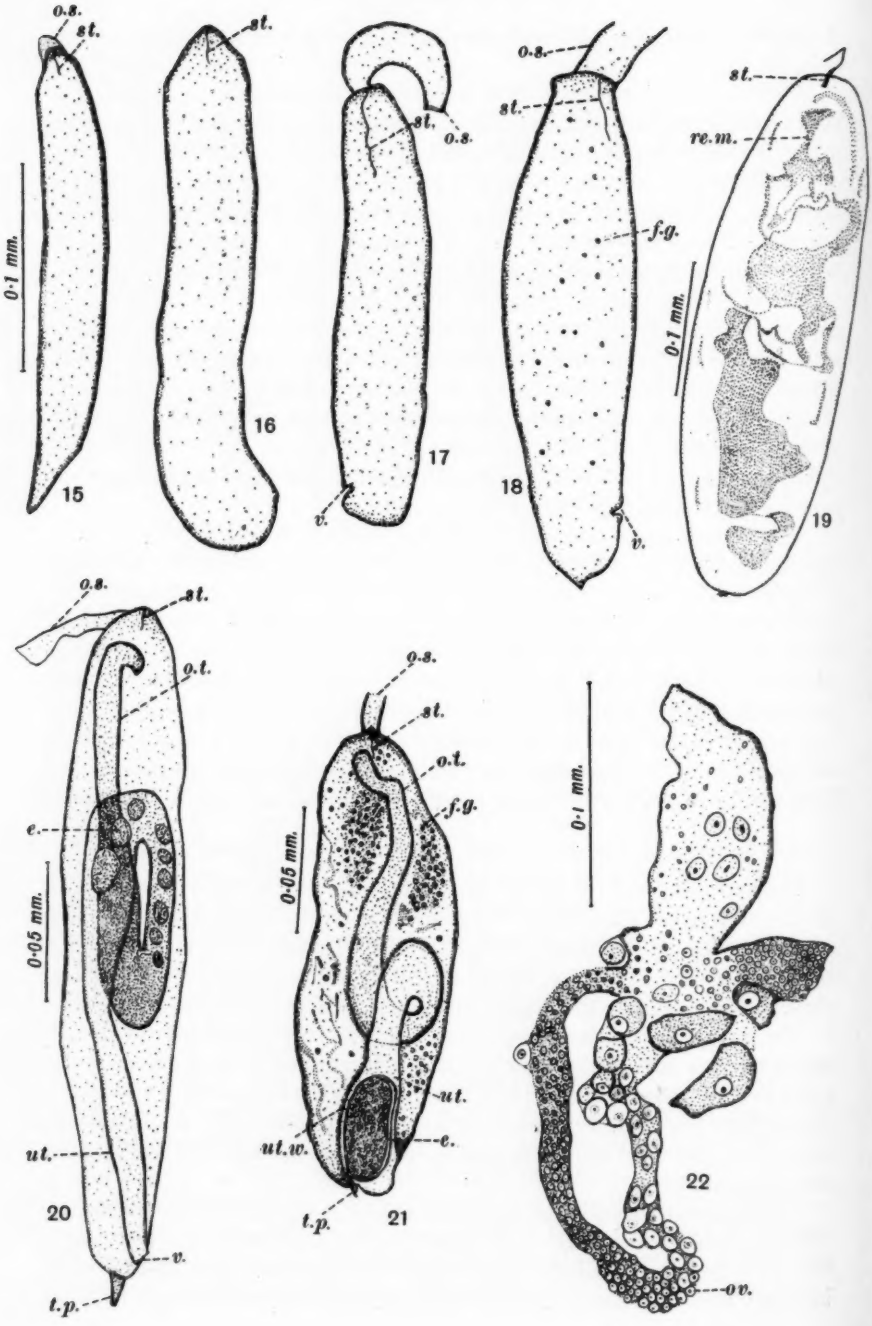
The relationship of this worm is difficult to establish with certainty as the free-living stages are unknown. Whether this male and the other larval stages in thrips belong to the same or to two different species is impossible to say until further light is thrown on its free-living stages. But since the males are found along with other forms, in most dissections, it appears to be associated with the nematode worm. In no case have these males been found passing out through the anus, nor have they been found in the rectum. This indicates that they remain in the host, probably fertilise the females in the early stages, which are so worm-like in appearance, and after fertilisation assume the degenerate form when mature. The life history of the parasitic female also indicates this.

(iv) *Female.*

The early stages of the immature parasitic female (Fig. 14) resemble those of the first larval form, hence they are difficult to differentiate. Slightly advanced stages of the female larvae (Figs. 15–18) may be distinguished by their greater size and peculiar behaviour. Measurements from eight individuals show how they widen and shorten as the female undergoes further development.

The larvae taper at both ends. The head is broadly rounded and is not separated by a constriction from the body. It is provided with a short stylet and a long oesophagus opening into the intestine. The body is filled with much reserve fatty material. The second larvae seem less active than the first larval forms, they moult several times, and in some cases the cast skin can be seen attached anteriorly.

After several ecdyses, the mature female stage is reached (Figs. 19–21). This form is highly degenerate and shows no evident relationship to its younger forms. It is thin, cylindroid, sac-shaped, 266–316μ long and 66–83μ wide, brownish with clear areas owing to the distribution of much reserve material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internally. Anteriorly a short stylet-shaped structure is seen. A trace of ovarian tube may be seen occasionally in fresh preparations, otherwise nothing is visible owing to dense fat globules.

The anterior portion is broader than the posterior, which ends in a short pointed tail (Figs. 20, 21). A clear hollow cuticular structure, sometimes of great length, is attached anteriorly. The short stylet is only visible in fresh preparations or in Lactophenol medium. In fixed and stained preparations the stylet is obscured. Alimentary canal and anus absent. In some specimens a trace of degenerate oesophagus may be seen with difficulty.

The reproductive organs (Figs. 20, 21) are well developed. The ovary is a long tubular structure extending to the anterior end of the body, looping posteriorly and widening out to form a uterus which opens to the outside by a small aperture; the vulva lies by the side of the tail papilla.

The reproductive system, when dissected out from the sac-shaped parasitic female, consists of a chain of ova in various stages of growth (Fig. 22). The ovarian tube widens out posteriorly and contains mature ova. Judged from the many ova seen in the tube the female must be very prolific. The worm is oviparous.

V. Biology.

(i) *Local distribution.*

Of the *Aptinothrips rufus* collected in ten localities in the vicinity of Edinburgh (King's Park, Blackford Hill, Morningside Fields, Braids, Liberton, Gifford, Boghall, Aberlady and Gullane (E. Lothian), and Forth Bridge (W. Lothian) only those from two areas[1] in King's Park were found to be infested with nematodes.

This localised distribution may depend on the nature of the soil.

(ii) *Seasonal distribution.*

Infested thrips were found throughout the year, monthly examinations being made from December, 1929, to November, 1930.[2] An approximate count of eggs, larvae and females was made to determine the average degree of infection. The results are recorded in Table I.

The figures show that infection was lowest in spring, highest in summer, intermediate in winter and autumn.

[1] (1) A small patch of land on the left side of Dunsapie Loch, (2) on the left opposite the bridge on Duddington Loch road.

[2] The average infection of *Aptinothrips rufus* by *Tylenchus aptini* for 1929–30 was 25·6 per cent.

Explanation of Text-figs. 15–22.

Figs. 15–18. Parasitic female developmental stages as found in host's body cavity.
Fig. 19. Parasitic female, mature, as seen in a drop of water. (Drawn 5 min. after dissection.)
Fig. 20. Parasitic female, mature, showing internal anatomy. (Carbol-lactic acid medium.)
Fig. 21. Parasitic female, mature, showing distribution of fat, reproductive organs and a ripe egg in the uterus about to pass out. Stylet seen anteriorly. (Carbol-lactic acid medium.)
Fig. 22. Ovary of parasitic female as dissected out. (Haematoxylin-eosin stain.)

(For lettering see Explanation of Figs. 1–14, p. 270.)

Table I. *Recording the number of thrips examined and found infected. Average seasonal figures.*

Winter		Spring		Summer		Autumn	
Exam.	Inf.	Exam.	Inf.	Exam.	Inf.	Exam.	Inf.
578	135	377	81	415	133	435	114
	23·3 %		21·4 %		32·0 %		26·2 %

The average percentage of infection according to months (see Chart 1) varies. The lowest infection (12·3 per cent.) was in March, the highest (37·5 per cent.) in July.

The lowest degree of infection (4 per cent.) observed on any one day in the year was on March 27th and the highest (55·5 per cent.) on October 7th, these figures are, however, based on only a small material.

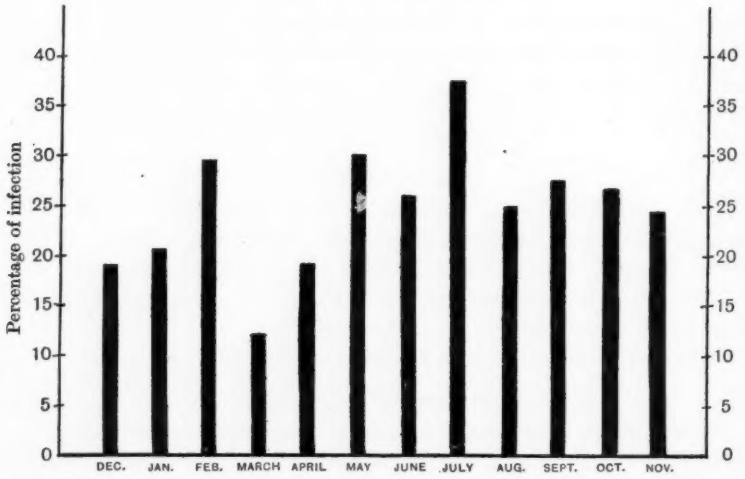


Chart 1. Average monthly infection of *Aptinothrips rufus* by *Tylenchus aptini*.

The highest number of eggs found in one thrips was 215, of larvae 255, of females 8, the lowest number being a single female nematode. The proportion of males (form 2) to the other stages of the nematodes in various counts made in October and November varied as follows: 27:228—4:43—6:18—4:38—11:73.

(iii) *Effect on host.*

Historical. The presence of parasitic nematodes in the body cavity of insects may have a profound effect on the host in some cases and practically no effect in others. Thus Uvarov (1928) records an epidemic in locusts due to parasitic nematodes. Grasshoppers artificially infested with one or two nematodes fail to develop gonads. According to Fuchs (1915), *Tylenchus contortus* diminishes the fecundity of the female host to about half in most cases, and occasionally kills its host. Wülker (1923, p. 464) mentions that Allantonema, parasitic in Hyloibus, has very little effect on the host, as he frequently found infested beetles in copulation

and having ripe reproductive organs. But he found that *Atractonema gibbosum* (p. 465), parasitic in *Cecidomyia pini* larvae, kills the host or delays its metamorphosis. *Sphaerularia bombi*, parasitic in queen bumble bees, is stated to cause sterility, as the parasitised queens are unable to form new colonies.

According to Cobb (1921, p. 669), *Howardula benigna*, parasitic in Diabrotica, reduces its size and weight and in cases of heavy infestation reduces fecundity. There is no suppression of the ovary, as he found 10,000–20,000 nematode larvae deposited with the beetle's eggs. Zur Strassen (1892, p. 7) found that 10–20 Bradynema has no effect on *Aphodius fimetarius*, as the gonads of the female can reach maturity. Recently Goodey (1930) has found that *Tylenchinema oscinellae*, parasitic in frit-fly, causes sterility in both sexes.

In Thysanoptera, Uzel (1895, p. 367) found that the presence of nematodes in *Thrips physopus* reduces the ovary, but he gives no detailed information.

The author's observations.

The presence of nematodes has no apparent effect on the external characters of *Aptinothrips rufus*, although the ovaries, as shown by reference to Figs. 23 and 24, become degenerated.

Fig. 23 represents the normal internal genital organs. The ovary consists of eight ovarioles arranged in groups of two. Terminally the ovarioles of both sides are attached together and these join with the hind end of the short salivary glands. The ovarioles are paniostic and three chief divisions are distinguished: (1) terminal filament, (2) germarium, (3) vitellarium. From a few to a large number of developing oocytes are found in the ovarioles which open by a short duct into the common oviduct. Posteriorly the oviduct is continuous with the vagina. The oblong, flat, retort-shaped receptaculum seminis opens by a duct into the vagina.

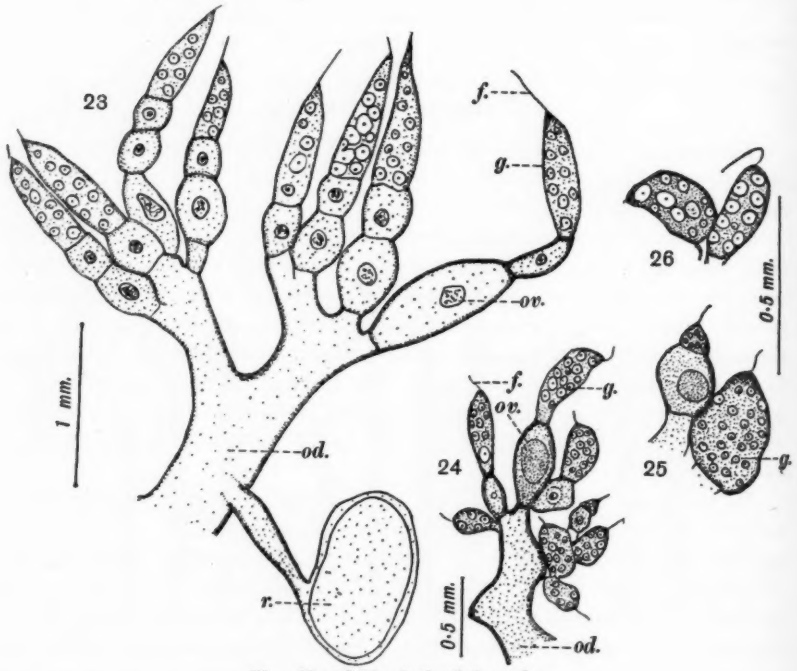
In parasitised thrips (Fig. 24) the ovary as a whole degenerates, the insect being incapable of producing eggs. All the ovarioles may be degenerate and be represented only by a short thin germarium having terminal filaments. The vitellarium in such cases is absent or a few ovarioles may show a trace of vitellarium. Where heavy infestation exists the ovary becomes attenuated and filamentous or may not be seen during dissection; the oviduct, vagina and receptaculum seminis are much reduced in size, whilst the receptaculum seminis may be present or absent. The other internal structures, including the salivary glands, were found unaffected, except in a few severely infested thrips which appeared sluggish, where the intestine was found much attenuated and very fragile.

Goodey (1930, p. 333) found that in a few cases the presence of two to four Tylenchinema in the abdominal cavity of the frit-fly did not appreciably affect the gonads, for they appeared normal; in these cases the worms were degenerate, small, and only partly developed. In *Aptinothrips rufus* I observed a few cases where but a few eggs and larvae, only one or two larvae and a few female worms were present, the insects' ovaries as well as the nematodes appearing normal. Whenever larvae were found with females and eggs in large numbers, the degeneration of ovary was certain and evident.

It appears in Aptinothrips that the nematodes always got the upper hand in suppressing the development of the ovary. The male insect is not attacked.

(iv) *Mode of infection.*

The larvae are motile and have been found wriggling about in the thoracic and abdominal cavity. When the first-form larvae reach a certain stage of development they enter the intestine and pass out through the anus. Schneider



Figs. 23 and 24. *Aptinothrips rufus.*

Fig. 23. Normal ovary.

Figs. 24–26. Degenerate ovary of parasitised insect.

Fig. 24. Ovary with some ovarioles having vitellarium.

Fig. 25. Two ovarioles from the same ovary more highly magnified.

Fig. 26. Ovary represented by germarium only.

g. germarium; *od.* oviduct; *ov.* ovum; *f.* terminal filament; *r.* receptaculum seminis.

(1885) and Wülker (1923) have found the larvae of *Sphaerularia bombi*, Allantonema and Bradynema taking a similar course. Goodey (1930) observed the same with Tylenchinema in the frit-fly. Cobb (1921), however, found that the larvae of Howardula pass out with the egg. None of the foregoing authors have actually seen the escape of the nematode from the abdominal cavity.

I have fortunately observed the process twice: The first-form larva, when

mature, bores by actively applying its slightly protruded stylet against the wall of the insect's mid-gut, whereby its head enters the lumen of the gut and the body of the larva follows quickly with a characteristic wriggling movement.

Once inside the gut the worm wanders down to the pyriform rectum, where occasionally several larvae are found and it appears that they remain there for some time before passing out. Larvae have never been observed either in the fore-gut, the anterior bulbous portion of the mid-gut, or the malphigian tubes. The whole process of boring and passing on to the rectum takes place in but a few seconds. It has been further observed that the nematodes are unable to pass through the rectal wall. Males have not been found in the rectum or issuing from the anus.

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ON THE WATER RESERVOIR OF A HORSE-CHESTNUT TREE.

By D. KEILIN.

From the Molteno Institute, University of Cambridge.

(With Plate XIII.)

In a previous paper (1927) I have given a general account of Dipterous larvae and their parasites living in the wounds of a horse-chestnut tree standing on the Downing site, Cambridge. This tree contained two types of wounds: (1) a surface wound with sap exudation or slime-flux, and (2) a large hollow or reservoir filled with water.

(1) The sap exudation harboured the larvae of *Dasyhelea obscura* Winnertz, *Rhyphus fenestralis* Scop., *Mycetobia pallipes* Meigen, and *Aulacogaster rufitarsis* Meigen.

(2) In the hollow of the tree there were two kinds of larvae: (*a*) aquatic larvae comprising *Anopheles plumbeus* Steph., *Aëdes geniculatus* (Olivier), and *Orthopodomyia pulchripalpis* Rond.[1] found in the water of the reservoir; and (*b*) larvae living in the wood pulp above the water level comprising those of *Rhipidia ctenophora* Loew, *Pericoma* sp., *Systenus adpropinquans* Loew, *Systenus sholtzi* Loew, *Homalomyia* sp., *Phaonia cincta* Zett., and *Phaonia keilini* Collin.

Some of these larvae have been found to harbour either a symbiont or new parasites, the structure and life histories of which have been described in detail. Thus, all the larvae of *Dasyhelea* contained in their perivisceral cavity a hereditary bacterial symbiont. Some of the larvae harboured also various parasites such as *Monosporella unicuspidata* Keilin, *Allantocystis dasyhelei* Keilin, *Helicosporidium parasiticum* Keilin. The larvae of *Systenus* harboured *Schisocystis legeri* Keilin, *Dendrorhynchus systeni* Keilin, *Lipotropha macrospora* Keilin, *Lipotropha microspora* Keilin, and finally the larvae of *Rhipidia* were often parasitised by a coccidian which has not yet been described.

For several years (1919–26) this tree supplied me with interesting material for the studies of the new life histories of Diptera and new forms of protists parasitic in their larvae. The study of this fauna came to an end when this tree with several others was cut down to make room for the new building of the Department of Pathology.

When the tree was cut, a portion of the trunk, about 6 ft. long, containing the reservoir was secured, and in June, 1926 was removed to a portion of the ground adjoining the south side of the Molteno Institute. On this ground and

[1] The early stages of this mosquito have been carefully described recently by P. Tate (1932).

about 70 yards away from its original position, the trunk was put upright with the opening of the reservoir facing south. The reservoir was filled with rain water but unfortunately a few days later it was found completely empty, the water having drained away through the central portion of the trunk, which was partly decayed and therefore porous. After several unsuccessful attempts to keep the rain water in the reservoir, the trunk with its reservoir was abandoned, but left standing for a whole year. On July 5th, 1927, the bottom of the reservoir was covered with a layer of cement which was allowed to dry and the reservoir was filled again with rain water. This time the water was retained, and soon acquired the characteristic tea-brown colour it always had before the tree was cut down. Three or four weeks later the water already contained a rich population of mosquito larvae which have been identified as those of *Anopheles plumbeus*, *Aëdes geniculatus*, and *Orthopodomyia pulchripalpis*: *i.e.* the same forms which were present in the reservoir of the living tree. On the other hand, the slime-flux with its fauna disappeared completely soon after the tree was cut down. From 1927 until now (1932) the water fauna was regularly examined and was always found to contain various stages of these mosquitoes: eggs, larvae, pupae, and often above the water numerous adult forms of mosquitoes.

During these five years, rain water was added to the reservoir each summer in order to compensate for the loss due to evaporation. On one occasion, in the summer of 1929, the reservoir was accidentally emptied, as the trunk had to be moved 2 yards nearer the building owing to the reconstruction of a road. The tree was then turned 180° and left standing with the reservoir facing north; it was again filled with rain water and the mosquito fauna soon reappeared.

The continuity of the mosquito fauna in the living tree and its dead trunk suggests that the fresh rain water added to the reservoir rapidly acquires the properties it had in the living tree. In fact, the general appearance of the water, its transparency, colour, absence of putrefaction, during the five years after the tree was cut remained the same as in the living tree. Moreover, the *p*H of the water during these years, first estimated colorimetrically and later with quinhydrone or hydrogen electrode remained between 9 and 9·3.

It is important to mention here that the relationship between the mosquito fauna and the *p*H of water, which is so often discussed in entomological literature, was for the first time pointed out by MacGregor in connection with the fauna of tree holes. Having found the larvae of *Anopheles plumbeus*, *Aëdes geniculatus*, and *Orthopodomyia pulchripalpis*, in the very acid water (*p*H 4·4) of a beech tree reservoir in Epping Forest, he concluded that the water of tree holes is usually acid and that the larvae of the above three species are adapted to the acid water. Later (1929), referring to my observations on the horse-chestnut fauna, he wrote "Keilin (1927) has reported the discovery of water in a horse-chestnut tree hole at Cambridge having a *p*H of 9·1. This high *p*H index is certainly abnormal."

This conclusion is not supported by my observations, as it is hardly possible to describe a *p*H of water which for more than ten years remains at about the same level, namely between 9 and 9·3, as "abnormal." Moreover, throughout this time the water always contained numerous larvae and pupae in all stages of development of the above-mentioned three species of mosquitoes. The fact that the same three species have been found by MacGregor in a beech tree with very acid water shows only that these mosquito larvae can live equally well in water of *p*H ranging between 4·4 and 9·3; in other words, that the *p*H of the water has no direct effect on the mosquito larvae.

While studying the fauna of this horse-chestnut tree, I carefully examined other trees standing on the Downing site but failed to find a tree hollow filled with water. As our tree trunk was standing from June, 1926 till July, 1927 completely empty and dry, it was somewhat difficult to explain the rapid repopulation of the reservoir when it was made to hold water. A satisfactory explanation of this phenomenon is now given by T. T. Macan and T. G. Tutin in their note which follows this paper. They have found, in fact, rot holes containing water and the same mosquito larvae in two horse-chestnut trees standing only 250 yards away from the Molteno Institute.

As far as I know the account of the fauna of this horse-chestnut tree given in this and previous papers deals with the first case of a tree the fauna of which has been followed for several years before and several years after it was cut down.

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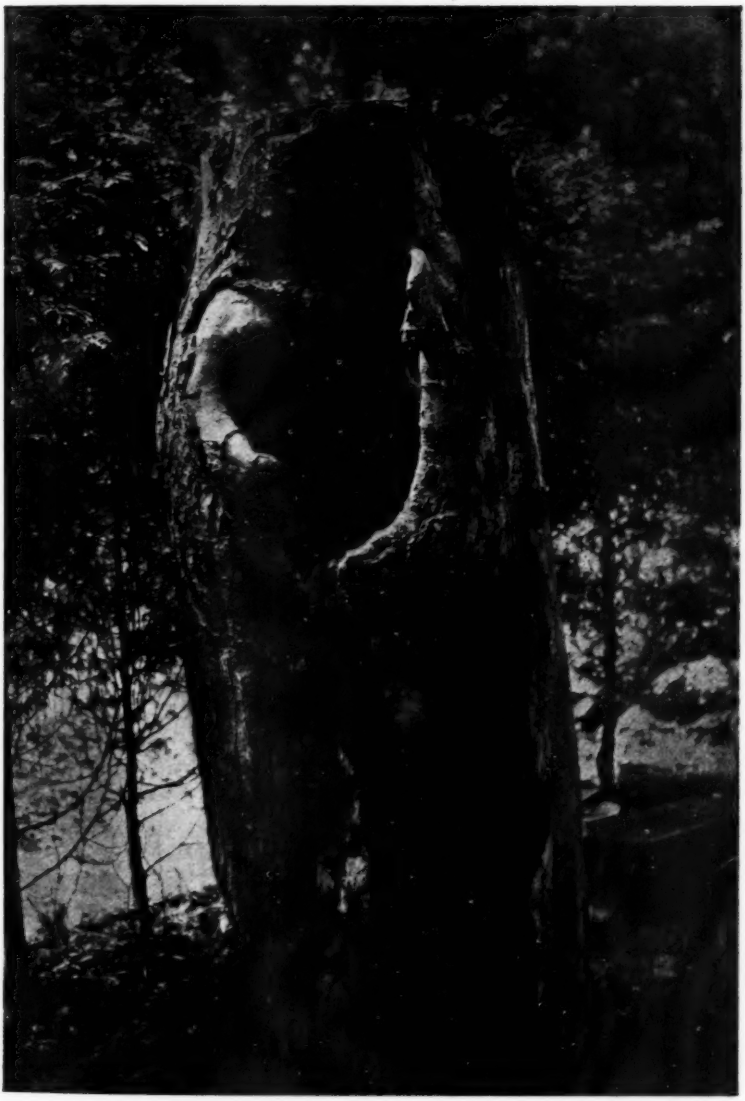

Trunk of horse-chestnut tree showing the water reservoir described in the text.

A NOTE ON ROT-HOLES IN HORSE-CHESTNUT TREES.

By T. T. MACAN (*Department of Zoology, University of Cambridge*) and T. G. TUTIN, B.A. (*Botany School, University of Cambridge*).

In 1927 Keilin described the fauna of a rot-hole and a slime-flux in a horse-chestnut tree (*Aesculus hippocastanum*) standing on the Downing site, Cambridge. For several consecutive years prior to 1927 he took there the larvae of the mosquito *Orthopodomyia pulchripalpis* Rondani (described as *O. albionensis* by MacGregor), this being only the second record for Britain. In 1926 the chestnut tree was cut down and the part of the trunk containing the rot-hole was placed standing on the ground close to the Molteno Institute on its south side. As the rot-hole, after a time, ceased to be water-tight, its bottom was cemented and the "reservoir" kept filled with water. (For further details see Keilin in this issue, p. 280.) Three to four weeks later larvae of *Orthopodomyia* were found in the reservoir. It was not clear how this recolonisation had taken place as no other breeding place was known in the vicinity.

Early in November, 1931, two rot-holes were noticed in chestnut trees in Downing College Fellow's Garden, about 250 yards southward from the original "reservoir." (*a*) The more distant of these contained larvae of the two common rot-hole mosquitoes, *Aëdes* (*Finlaya*) *geniculatus* (Olivier) and *Anopheles plumbeus* Stephens, besides a number of *Orthopodomyia* larvae. Most of these were in an early instar, but there were a few in the fourth. (*b*) The other hole, apparently similar to (*a*), contained only a few young larvae of *A. plumbeus*.

Rot-hole (*a*), in which *Orthopodomyia* occurred, was some 20 ft. from the ground, on the north-west side of the tree. The opening was oval, and measured about 4 by 6 in. The hole sloped toward the centre of the tree, and then ran horizontally a little way. The reservoir was about two-thirds full; the depth of the water was only 3 in. and its total volume slightly under a litre. The bottom of the reservoir was covered by a deposit of decomposed wood pulp. The water itself was quite clear and of a brown colour with a *p*H of about 8·5.

Rot-hole (*b*), also facing north-west and about 12 ft. from the ground, had a circular opening of about 3 in. It sloped straight in, and as before had the reservoir about two-thirds full with water 3 in. deep. The volume was a little less than in hole (*a*), while the colour and transparency were the same. The *p*H was 7·4.

It seems very probable that it was by mosquitoes bred from one or both of these rot-holes that the original reservoir was so rapidly recolonised.

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THE OCCURRENCE OF PECULIAR BODIES IN BLOOD CELLS OF ANIMALS AFTER TREATMENT WITH SOME CHEMICAL COMPOUNDS.

By P. TATE and M. VINCENT.

From the Molteno Institute for Research in Parasitology, University of Cambridge.

(With 1 Figure in the Text.)

While engaged in testing the possible antimalarial properties of numerous chemical compounds on avian malaria[1] (*Plasmodium relictum* Grassi and Feletti 1891) peculiar bodies which stained densely with Leishman's stain were noticed in the erythrocytes of some of the birds. Further work showed that these bodies were produced by treating the birds with two of the compounds used for antimalarial tests. The compounds are: R. 59, aminopropylamino-styrylmethoxyquinoline trihydrochloride, prepared by Prof. Robinson of Oxford; and P. 25, *o*-β-diethylaminoethyl harmol hydrochloride, prepared by Prof. Pyman. The compounds were given to the birds in aqueous solutions and were fed directly into the stomach by the catheter-tube method described by Roehl (1926), the doses being calculated in mg. per 20 grm. of body weight of bird.

In the erythrocytes the bodies are very distinct and appear in blood films stained by Leishman's method as sharply delimitated dark blue bodies scattered in the cytoplasm. They are variable in size and shape, although they are mostly ovoid or spherical. The size may show all gradations from scarcely perceptible specks to spherical bodies nearly 1μ in diameter (Fig. 1 *A–D*). The bodies produced by the compound R. 59 appear to be smaller than those produced by P. 25 (Fig. 1 *E*, *F*). The number of bodies present in an erythrocyte varies from one to as many as seven or even more, and they may occur scattered singly throughout the cytoplasm or arranged in one or more closely packed groups. In the same erythrocyte, or even in the same group, the bodies may vary greatly in size.

These bodies are always found in the blood of birds after treatment with the two compounds whether the birds so treated are normal birds or are suffering from acute or chronic malarial infections. The two compounds differ in the quantities which must be administered to induce the development of the bodies in the blood. The maximum tolerated dose of each compound for

[1] This work was done on behalf of the Chemotherapy Committee of the Medical Research Council.

canaries is 5 mg. per 20 grm. of body weight; sometimes this dose may be given daily for 6 consecutive days, but some birds will not tolerate repeated doses of more than 2·5 or 1·25 mg. With compound R. 59 one dose of 2·5 mg. is not sufficient to produce any bodies, but repeated daily doses of 5 mg. or 2·5 mg. causes the appearance of the bodies in the blood in 3 or 4 days, and they persist in the blood, in gradually decreasing numbers, for about 4 days after the last dose. With compound P. 25 one dose of 2·5 mg. is sufficient to produce bodies in the blood within 17 hours and they persist for 3 days: with larger (5 mg.), or repeated, doses the bodies may continue in the blood as long as 10 or 11 days after the last dose. With this compound subcutaneous injection of 1·25 mg. for 3 days produced bodies in 24–48 hours which persisted

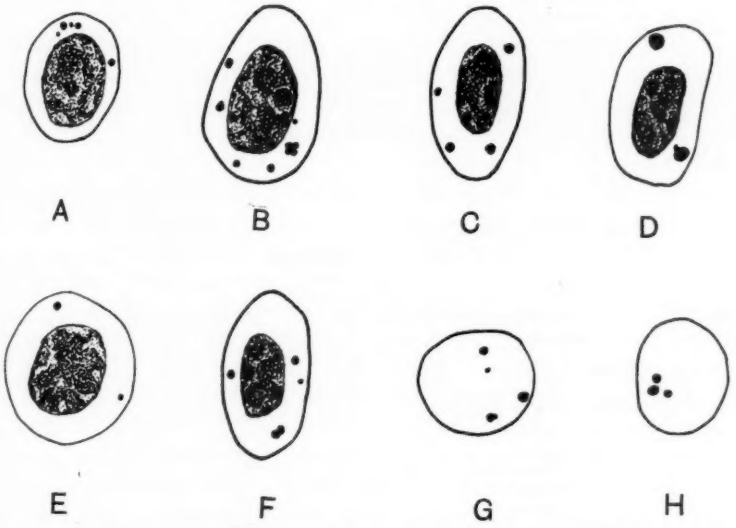

Fig. 1. Bodies in blood cells of canaries and mice treated with the compounds R. 59 and P. 25. *A–D*, blood cells of canary treated with P. 25; *A*, *B*, immature, *C*, *D*, mature, erythrocytes. *E*, *F*, erythrocytes of canary treated with R. 59; *E*, immature, *F*, mature, erythrocytes. *G*, *H*, polychromatic erythrocytes of mouse treated with P. 25. All × 2500.

for 5 or 6 days. Intramuscular injection of one dose of 0·6 mg. failed to produce bodies, but the compound was too irritating to increase the dose or to repeat it. With compound R. 59 intramuscular injection of 0·6 mg. gave no result; and subcutaneous injection of 1·25 mg. on 5 consecutive days gave doubtful results, but the experiment was not carried on longer or repeated owing to the irritant action of the compound when injected. Details of the experiments done on canaries with both compounds are given in Tables I and II.

Careful examination of blood films and of smears from various organs showed that these bodies are also produced in other blood cells besides the erythrocytes, and they were seen in eosinophiles, leucocytes and reticulo-

endothelial cells. They first appear in immature erythrocytes and in eosinophiles.

Post-mortem examination of birds with very numerous bodies in the blood

Table I. A. *Aminopropylaminostyrylmethoxyquinoline trihydrochloride.*

		Doses given			Bodies seen in blood	
Bird	Previous history	No. of days	Each dose mg.	Method	Earliest	Latest
1	Acute malaria	6	5	Oral	6	18 (K.)
2	Acute malaria	4	5	Oral	4	7 (D.)
3	Chronic malaria	2	2·5	Oral	3	7
		2	1·25	Oral	—	—
4	Chronic malaria	1	2·5	Oral	None	—
5	Chronic malaria	1	0·6	Intramus.	None	—
6	Chronic malaria	5	1·25	Subcut.	5	5
7	Chronic malaria	1	2·5	Oral	None	—
8	Normal	5	5	Oral	5	5 (D.)
9	Normal	4	2·5	Oral	3	7
9 *a*	No. 9, re-treated	1	5	Oral	4	8
		4	2·5	Oral	—	—
	Again treated starting 14th day	5	2·5	Oral	18	21
9 *b*	No. 9 3rd treatment	9	2·5	Oral	6	10
10	Normal	4	2·5	Oral	—	—
		1	1·25	Oral	4	10

Table II. B. *o-β-diethylaminoethyl harmol hydrochloride.*

		Doses given			Bodies seen in blood	
Bird	Previous history	No. of days	Each dose mg.	Method	Earliest	Latest
1	Acute malaria	6	5	Oral	? 5	18
2	Chronic malaria	2	5	Oral	2	14
		1	2·5	Oral	—	—
3	Chronic malaria	1	5	Oral	1	18
		4	2·5	Oral	—	—
3 *a*	No. 3 re-treated	9	2·5	Oral	17 hours	10 ?
4	Chronic malaria	1	5	Oral	1	7
4 *a*	No. 4 re-treated starting 14th day	5	2·5	Oral	15	30
5	Chronic malaria	1	2·5	Oral	1	3
6	Normal	3	5	Oral	2	14
7	Normal	5	2·5	Oral	1	9 (K.)
8	Chronic malaria	1	0·6	Intramus.	None	—
9	Chronic malaria	3	1·25	Subcut.	2	6
10	Chronic malaria	3	1·25	Subcut.	1	5

Explanation of Tables I and II. In column 3 the figures represent the number of consecutive days on which doses were given; in column 4 the amount of compound given in each dose is represented in mg. per 20 grm. of body weight; in column 5 the mode of administering the compound is stated: Intramus., intramuscular; Subcut., subcutaneous. In column 6 is given the number of days after first dose on which bodies were seen in the blood; in column 7 is given the last day on which the bodies were seen: ? 5, blood not examined before 5th day; 10 ?, blood not examined after 10th day. (D.), bird died; (K.), bird killed.

cells did not reveal any pathological changes in the organs. Fixation of wet smears and portions of the organs, liver, spleen and kidney, with fluids such as those of Schaudinn, Bouin, Flemming and Duboscq-Brazil, and staining with the usual cytological stains did not differentiate the bodies. In freshly drawn

blood they are not visible under the microscope, even with dark ground illumination. In smears and blood films the bodies stain in the same way with both Giemsa's and Leishman's stains. They are also easily recognisable in the blood cells in sections of organs fixed in Tellyesnicky's and Bouin's fluids and stained by the slow Giemsa method, and have the same appearance as in smears and blood films.

Renewed treatment of birds after the bodies have ceased to be visible in the blood results in the reappearance of the bodies which follow the same course as in birds not previously treated. Inoculation of two clean birds with blood from birds showing numerous bodies as a result of treatment with each of the compounds R. 59 and P. 25 gave no evidence that they can be transmitted by blood inoculation. An attempt to produce the bodies *in vitro* by mixing freshly drawn blood and normal saline-citrate solution with a solution of P. 25 and making smears at intervals failed to show them within 24 hours.

Subcutaneous injection of a mouse with 1·25 mg. of compound P. 25 resulted in the appearance within 24 hours of bodies similar to those seen in canaries (Fig. 1 *G*, *H*). They increased in number with repeated injections and persisted in the blood 2 days after the last injection. In the mouse they were seen only in polychromatic erythrocytes. Similar injection into a mouse of 1·25 mg. of R. 59 for 5 consecutive days resulted in the appearance of a few of the bodies in the blood cells on the seventh and eighth days; but owing to its irritant action longer treatment with this compound was not attempted.

In our opinion the evidence is strongly against the possibility that these bodies are living organisms. This view is supported by the facts that they stain uniformly and show no cytological differentiation; they have not been seen to undergo fission or to multiply in the cells; they cannot be transmitted by blood inoculation; they are not visible by direct microscopical examination, even with dark ground illumination; and they do not appear in material subjected to the usual cytological fixatives and stained. In their staining reactions and microscopical appearance they are easily differentiated from other enigmatic cell inclusions found in the blood with which we have compared them, including *Bartonella*, *Grahamella*, anaplasmata and Jolly bodies. The difference from Jolly bodies was readily seen in the mice treated with R. 59 and P. 25 as both were present in the same blood films, and the dark blue stain of these new bodies contrasted strongly with the deep red of the Jolly bodies. Dias and Aragão (1914) have produced anaplasmata in the blood of various animals by the injection of some chemical substances such as phenylhydrazin and nitrobenzol. This was confirmed by Laveran and Franchini (1914), who refer to the bodies as "marginal points." It appears, however, that anaplasmata and Jolly bodies are morphologically indistinguishable. Comparison of the bodies produced by R. 59 and P. 25 with stained slides of *Anaplasma marginale*, from Prof. Brumpt, showed that they are not the same: while they stain dark blue, *A. marginale* stain deep red, with Giemsa's stain.

In our opinion these bodies are non-living inclusions, deposits, or local

alterations in the cytoplasm of the blood cells due to the action of the compounds R. 59 and P. 25.

SUMMARY.

Oral and subcutaneous administration of the compounds aminopropylaminostyrylmethoxyquinoline trihydrochloride and *o*-*β*-diethylaminoethyl harmol hydrochloride causes the appearance of peculiar bodies in the blood cells of canaries and mice.

The bodies are irregular in shape and size, stain intensely blue with Leishman's and Giemsa's stains, and are most numerous in erythrocytes, but occur also in eosinophiles, leucocytes and reticulo-endothelial cells.

The bodies show no cytological structure, are not seen to undergo multiplication, and are not transmissible by blood inoculation.

They are apparently non-living inclusions in the blood cells caused by the action of these two chemical compounds.

We wish to express our thanks to Dr H. P. Bayon for his kind help and the loan of stained preparations of *Bartonella*, *Grahamella* and Jolly bodies.

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